10/501,629.
no Patent No: 6,919,186

Attachment 2

US-09-213-343-4
; Sequence 4, Application US/09213343
; Patent No. 6316252
; GENERAL INFORMATION:
; APPLICANT: Harms, Jerome S.
; APPLICANT: Splitter, Gary A.
; TITLE OF INVENTION: Biotherapeutic Delivery System
; FILE REFERENCE: 960296.95564
; CURRENT APPLICATION NUMBER: US/09/213,343
; CURRENT FILING DATE: 1998-12-17
; NUMBER OF SEQ ID NOS: 4
; SOFTWARE: PatentIn Ver. 2.0
; SEQ ID NO 4
; LENGTH: 238
; TYPE: PRT
; ORGANISM: Aequorea victoria
US-09-213-343-4

Query Match 93.7%; Score 1195; DB 2; Length 238;
Best Local Similarity 92.4%; Pred. No. 4e-124;
Matches 220; Conservative 8; Mismatches 10; Indels 0; Gaps 0;

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Qy         61 VTTLSYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFFEDDGNYKSRAEVKFEGDTLV 120
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Qy        181 HYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMIYFGFVTAAAITHGMDELYK 238
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RESULT 3
US-09-967-301-3
; Sequence 3, Application US/09967301
; Patent No. 6919186
; GENERAL INFORMATION:
; APPLICANT: Stubbs, Simon L.
; APPLICANT: Jones, Anne E.
; APPLICANT: Michael, Nigel P.
; APPLICANT: Thomas, Nicholas
; TITLE OF INVENTION: Fluorescent Proteins
; FILE REFERENCE: PA0111
; CURRENT APPLICATION NUMBER: US/09/967,301
; CURRENT FILING DATE: 2001-09-28
; PRIOR APPLICATION NUMBER: GB 0109858.1
; PRIOR FILING DATE: 2001-04-23
; NUMBER OF SEQ ID NOS: 19
; SOFTWARE: PatentIn Ver. 2.1
; SEQ ID NO 3
; LENGTH: 238
; TYPE: PRT
; ORGANISM: Artificial Sequence
; FEATURE:
; OTHER INFORMATION: Description of Artificial Sequence: synthetic
; OTHER INFORMATION: protein
US-09-967-301-3

Query Match 93.7%; Score 1195; DB 2; Length 238;
Best Local Similarity 91.6%; Pred. No. 4e-124;
Matches 218; Conservative 11; Mismatches 9; Indels 0; Gaps 0;

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Qy        121 NRIELTGTDFKEDGNILGNKMEYNYNAHNVYIMTDKAKNGIKVNFKIRHNIEDGSVQLAD 180
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Db        181 HYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLGFVTAAGITHGMDELYK 238
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RESULT 4
US-10-471-857A-1
; Sequence 1, Application US/10471857A
; Patent No. 7015310
; GENERAL INFORMATION:

UniProt database.
10/501629
Attachment 1

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DE   Green fluorescence protein.
GN   Name=375Gfp;
OS   Azomonas agilis.
OC   Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
OC   Pseudomonadaceae; Azomonas.
OX   NCBI_TaxID=116849;
RN   [1]
RP   NUCLEOTIDE SEQUENCE.
RA   Koranyi P., Berenyi M., Burg K.;
RL   Submitted (NOV-2000) to the EMBL/GenBank/DDBJ databases.
CC   ---------------------------------------------------------------------
CC   Copyrighted by the UniProt Consortium, see http://www.uniprot.org/terms
CC   Distributed under the Creative Commons Attribution-NoDerivs License
CC   ---------------------------------------------------------------------
DR   EMBL; AF324405; AAN86137.1; -; Genomic_DNA.
DR   HSSP; P42212; 1BFP.
DR   SMR; Q8GHE4; 2-237.
DR   GO; GO:0008218; P:bioluminescence; IEA.
DR   GO; GO:0006091; P:generation of precursor metabolites and energy; IEA.
DR   InterPro; IPR009017; GFP_like.
DR   InterPro; IPR011584; GFP_related.
DR   InterPro; IPR000786; Green_fl_protein.
DR   Pfam; PF01353; GFP; 1.
DR   PRINTS; PR01229; GFLUORESCENT.
DR   ProDom; PD013756; Green_fl_protein; 1.
SQ   SEQUENCE   238 AA;  26902 MW;  15FE9B9C5B4F6B89 CRC64;

  Query Match             94.5%;  Score 1207;  DB 2;  Length 238;
  Best Local Similarity   92.9%;  Pred. No. 3e-90;
  Matches  221;  Conservative    9;  Mismatches    8;  Indels    0;  Gaps    0;

Qy          1 MSKGAELFTGVVPILIELNGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 60
              |||| |||||||||:||:|||||||||||||||||||||||||||||||||||||||||
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Qy         61 VTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKSRAEVKFEGDTLV 120
              ||||||||||||||||||:|||||||||||:|||||||||||||:||||||||||||
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Qy        121 NRIELTGTDFKEDGNILGNKMEYNYNAHNVYIMTDKAKNGIKVNFKIRHNIEDGSVQLAD 180
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Qy        181 HYQQNTPIGDGPVLLPDNHYLSTQSTLSKDPNEKRDHMIYFEFVTAAAITHGMDELYK 238
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Db        181 HYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK 238
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RESULT 3

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GFP_AEQVI
ID   GFP_AEQVI      STANDARD;      PRT;   238 AA.
AC   P42212; Q17104; Q27903;
DT   01-NOV-1995, integrated into UniProtKB/Swiss-Prot.
DT   01-NOV-1995, sequence version 1.
DT   25-JUL-2006, entry version 56.
DE   Green fluorescent protein.
GN   Name=GFP;
OS   Aequorea victoria (Jellyfish).
OC   Eukaryota; Metazoa; Cnidaria; Hydrozoa; Hydroida; Leptomedusae;
OC   Aequoreidae; Aequorea.
OX   NCBI_TaxID=6100;
RN   [1]
RP   NUCLEOTIDE SEQUENCE [MRNA], AND PARTIAL PROTEIN SEQUENCE.
RX   MEDLINE=92175527; PubMed=1347277; DOI=10.1016/0378-1119(92)90691-H;
RA   Prasher D.C., Eckenrode V.K., Ward W.W., Prendergast F.G.,
RA   Cormier M.J.;
RT   "Primary structure of the Aequorea victoria green-fluorescent
RT   protein.";
RL   Gene 111:229-233(1992).
RN   [2]
RP   NUCLEOTIDE SEQUENCE [MRNA].
RX   MEDLINE=94185810; PubMed=8137953; DOI=10.1016/0014-5793(94)80472-9;
RA   Inouye S., Tsuji F.I.;
RT   "Aequorea green fluorescent protein. Expression of the gene and
RT   fluorescence characteristics of the recombinant protein.";
RL   FEBS Lett. 341:277-280(1994).
RN   [3]
RP   NUCLEOTIDE SEQUENCE [MRNA].
RX   MEDLINE=97299832; PubMed=9154981; DOI=10.1023/A:1005740823703;
RA   Rouwendal G.J.A., Mendes O., Wolbert E.J.H., de Boer A.D.;
RT   "Enhanced expression in tobacco of the gene encoding green fluorescent
RT   protein by modification of its codon usage.";
RL   Plant Mol. Biol. 33:989-999(1997).
RN   [4]
RP   CHROMOPHORE.
RX   MEDLINE=93192221; PubMed=8448132;
RA   Cody C.W., Prasher D.C., Westler W.M., Prendergast F.G., Ward W.W.;
RT   "Chemical structure of the hexapeptide chromophore of the Aequorea
RT   green-fluorescent protein.";
RL   Biochemistry 32:1212-1218(1993).
RN   [5]
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RP   X-RAY CRYSTALLOGRAPHY (1.9 ANGSTROMS).
RX   MEDLINE=96355665; PubMed=8703075;
RA   Ormoe M., Cubitt A.B., Kallio K., Gross L.A., Tsien R.Y.,
RA   Remington S.J.;
RT   "Crystal structure of the Aequorea victoria green fluorescent
RT   protein.";
RL   Science 273:1392-1395(1996).
RN   [6]
RP   X-RAY CRYSTALLOGRAPHY (1.9 ANGSTROMS).
RX   MEDLINE=98294543; PubMed=9631087; DOI=10.1038/nbt1096-1246;
RA   Yang F., Moss L.G., Phillips G.N. Jr.;
RT   "The molecular structure of green fluorescent protein.";
RL   Nat. Biotechnol. 14:1246-1251(1996).
RN   [7]
RP   X-RAY CRYSTALLOGRAPHY (2.5 ANGSTROMS) OF MUTANT WITH YELLOW EMISSION.
RX   MEDLINE=98455509; PubMed=9782051; DOI=10.1016/S0969-2126(98)00127-0;
RA   Wachter R.M., Elsliger M.A., Kallio K., Hanson G.T., Remington S.J.;
RT   "Structural basis of spectral shifts in the yellow-emission variants
RT   of green fluorescent protein.";
RL   Structure 6:1267-1277(1998).
RN   [8]
RP   X-RAY CRYSTALLOGRAPHY (2.0 ANGSTROMS).
RX   MEDLINE=99238303; PubMed=10220315; DOI=10.1021/bi9902182;
RA   Elsliger M.A., Wachter R.M., Hanson G.T., Kallio K., Remington S.J.;
RT   "Structural and spectral response of green fluorescent protein
RT   variants to changes in pH.";
RL   Biochemistry 38:5296-5301(1999).
CC   -!- FUNCTION: Energy-transfer acceptor. Its role is to transduce the
CC       blue chemiluminescence of the protein aequorin into green
CC       fluorescent light by energy transfer. Fluoresces in vivo upon
CC       receiving energy from the Ca(2+)-activated photoprotein aequorin.
CC   -!- BIOPHYSICOCHEMICAL PROPERTIES:
CC       Absorption:
CC         Abs(max)=395 nm;
CC         Note=Exhibits a smaller absorbance peak at 470 nm. The
CC         fluorescence emission spectrum peaks at 509 nm with a shoulder
CC         at 540 nm;
CC   -!- SUBUNIT: Monomer.
CC   -!- TISSUE SPECIFICITY: Photocytes.
CC   -!- PTM: Contains a chromophore consisting of modified amino acid
CC       residues. The chromophore is formed by autocatalytic backbone
CC       condensation between Xaa-N and Gly-(N+2), and oxidation of Tyr-
CC       (N+1) to didehydrotyrosine. Maturation of the chromophore requires
CC       nothing other than molecular oxygen.
CC   -!- BIOTECHNOLOGY: Fluorescent proteins have become a useful and
CC       ubiquitous tool for making chimeric proteins, where they function
CC       as a fluorescent protein tag. Typically they tolerate N- and C-
CC       terminal fusion to a broad variety of proteins. They have been
CC       expressed in most known cell types and are used as a noninvasive
CC       fluorescent marker in living cells and organisms. They enable a
CC       wide range of applications where they have functioned as a cell
CC       lineage tracer, reporter of gene expression, or as a measure of
CC       protein-protein interactions.
CC   -!- SIMILARITY: Belongs to the GFP family.
CC   -!- WEB RESOURCE: NAME=Protein Spotlight; NOTE=Issue 11 of June 2001;
CC       URL="http://www.expasy.org/spotlight/back_issues/sptlt011.shtml".
CC   ---------------------------------------------------------------------
CC   Copyrighted by the UniProt Consortium, see http://www.uniprot.org/terms
CC   Distributed under the Creative Commons Attribution-NoDerivs License
CC   ---------------------------------------------------------------------
DR   EMBL; M62654; AAA27722.1; -; mRNA.
DR   EMBL; M62653; AAA27721.1; -; mRNA.
DR   EMBL; L29345; AAA58246.1; -; mRNA.
DR   EMBL; X96418; CAA65278.1; -; mRNA.
DR   PIR; JS0692; JQ1514.
DR   PDB; 1B9C; X-ray; A/B/C/D=2-238.
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DR   PDB; 1CV7; X-ray; A=-.
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DR   PDB; 2G6E; X-ray; A=-.
DR   LinkHub; P42212; -.
DR   GO; GO:0008218; P:bioluminescence; TAS.
DR   GO; GO:0006091; P:generation of precursor metabolites and energy; TAS.
DR   InterPro; IPR009017; GFP_like.
DR   InterPro; IPR011584; GFP_related.
DR   InterPro; IPR000786; Green_fl_protein.
DR   Pfam; PF01353; GFP; 1.
DR   PRINTS; PR01229; GFLUORESCENT.
DR   ProDom; PD013756; Green_fl_protein; 1.
KW   3D-structure; Chromophore; Direct protein sequencing; Luminescence;
KW   Photoprotein.
FT   CHAIN         1    238       Green fluorescent protein.
FT                                /FTId=PRO_0000192576.
FT   MOD_RES      66     66       (Z)-2,3-didehydrotyrosine.
FT   CROSSLNK     65     67       5-imidazolinone (Ser-Gly).
FT   VARIANT     100    100       F -> Y.
FT   VARIANT     108    108       T -> S.
FT   VARIANT     141    141       L -> M.
FT   VARIANT     219    219       V -> I.
FT   CONFLICT      2      2       S -> G (in Ref. 3).
FT   CONFLICT     25     25       H -> Q (in Ref. 2).
FT   CONFLICT     80     80       Q -> R (in Ref. 3).

  Query Match             94.4%;  Score 1205;  DB 1;  Length 238;
  Best Local Similarity   92.9%;  Pred. No. 4.3e-90;
  Matches  221;  Conservative    8;  Mismatches    9;  Indels    0;  Gaps    0;

Qy          1 MSKGAELFTGVVPILIELNGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTL 60
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Qy         61 VTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYIQERTIFFKDDGNYKSRAEVKFEGDTLV 120
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Db        121 NRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD 180

Qy        181 HYQQNTPIGDGPVLLPDNHYLSTQSTLSKDPNEKRDHMIYFEFVTAAAITHGMDELYK 238
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RESULT 4
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10/501,629

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=> d his

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     LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007
L1        115659 S GREEN (W)FLUORESCENT
L2            22 S AEQUOREA (W) COERULESCENS
L3            13 S L1 AND L2
L4             7 DUP REM L3 (6 DUPLICATES REMOVED)
L5             5 S "ACGFPL"
L6            20 S "E222G" AND L1
L7            12 DUP REM L6 (8 DUPLICATES REMOVED)
L8             4 S L2 AND L6
L9           302 S V11I OR K101E OR 1206A OR F64L
L10         3003 S AEQUOREA AND L1
L11           35 S L9 AND L10
L12           17 DUP REM L11 (18 DUPLICATES REMOVED)
L13           13 DUP REM L2 (9 DUPLICATES REMOVED)
                 E GURSKAYA N G/AU
L14          142 S E3-E7
                 E FRADKOV A F/AU
L15          112 S E3 OR E12
                 E LUKYANOV S A/AU
L16          201 S E3
                 E PUNKOVA N I/AU
L17            3 S E6
L18       269981 S L14 OR L15 OR 116 OR 117
L19            3 S L2 AND L18
L20            2 DUP REM L19 (1 DUPLICATE REMOVED)

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Connecting via Winsock to STN

Welcome to STN International! Enter x:x

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PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

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NEWS  1          Web Page for STN Seminar Schedule - N. America
NEWS  2  MAR 15  WPIDS/WPIX enhanced with new FRAGHITSTR display format
NEWS  3  MAR 16  CASREACT coverage extended
NEWS  4  MAR 20  MARPAT now updated daily
NEWS  5  MAR 22  LWPI reloaded
NEWS  6  MAR 30  RDISCLOSURE reloaded with enhancements
NEWS  7  APR 02  JICST-EPLUS removed from database clusters and STN
NEWS  8  APR 30  GENBANK reloaded and enhanced with Genome Project ID field
NEWS  9  APR 30  CHEMCATS enhanced with 1.2 million new records
NEWS 10  APR 30  CA/CAplus enhanced with 1870-1889 U.S. patent records
NEWS 11  APR 30  INPADOC replaced by INPADOCDB on STN
NEWS 12  MAY 01  New CAS web site launched
NEWS 13  MAY 08  CA/CAplus Indian patent publication number format defined
NEWS 14  MAY 14  RDISCLOSURE on STN Easy enhanced with new search and display
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NEWS 15  MAY 21  BIOSIS reloaded and enhanced with archival data
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NEWS 17  MAY 21  CA/CAplus enhanced with additional kind codes for German
                 patents
NEWS 18  MAY 22  CA/CAplus enhanced with IPC reclassification in Japanese
                 patents
NEWS 19  JUN 27  CA/CAplus enhanced with pre-1967 CAS Registry Numbers

NEWS EXPRESS  NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c, CURRENT
              MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
              AND CURRENT DISCOVER FILE IS DATED 25 SEPTEMBER 2006.

NEWS HOURS    STN Operating Hours Plus Help Desk Availability
NEWS LOGIN    Welcome Banner and News Items
NEWS IPC8     For general information regarding STN implementation of IPC 8
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Enter NEWS followed by the item number or name to see news on that specific topic.

* * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	1.47	1.47

FILE 'MEDLINE' ENTERED AT 10:20:39 ON 29 JUN 2007

FILE 'EMBASE' ENTERED AT 10:20:39 ON 29 JUN 2007

FILE 'BIOSIS' ENTERED AT 10:20:39 ON 29 JUN 2007

FILE 'BIOTECHDS' ENTERED AT 10:20:39 ON 29 JUN 2007

FILE 'SCISEARCH' ENTERED AT 10:20:39 ON 29 JUN 2007

FILE 'HCAPLUS' ENTERED AT 10:20:39 ON 29 JUN 2007

FILE 'NTIS' ENTERED AT 10:20:39 ON 29 JUN 2007

FILE 'LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007


```
=> s green (w)fluorescent
L1      115659 GREEN (W) FLUORESCENT

=> s aequorea (w) coerulescens
L2          22 AEQUOREA (W) COERULESCENS

=> s l1 and l2
L3          13 L1 AND L2

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4             7 DUP REM L3 (6 DUPLICATES REMOVED)

=> d 1-7 ibib ab

L4    ANSWER 1 OF 7  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-19750  BIOTECHDS
TITLE:            Novel transgenic zebrafish that expresses Tau, amyloid
                  precursor protein or presenilin polypeptide or their fusion
                  polypeptides, or amyloid beta polypeptide, useful for
                  identifying modulator of Alzheimer's disease;
                     tau protein, amyloid precursor protein, amyloid-beta or
                     presenilin gene transfer and expresion in zebrafish neuron
                     for zebrafish transgenic fish and drug screening
AUTHOR:           RUBINSTEIN A L
PATENT ASSIGNEE:  ZYGOGEN LLC
PATENT INFO:      WO 2006081539 3 Aug 2006
APPLICATION INFO: WO 2006-US3165 27 Jan 2006
PRIORITY INFO:    US 2005-647493 27 Jan 2005; US 2005-647493 27 Jan 2005
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2006-539425 [55]
AB    DERWENT ABSTRACT:
      NOVELTY - A transgenic zebrafish that expresses (a) a tau polypeptide,
      amyloid precursor protein (APP), amyloid beta or presenilin polypeptide,
```

comprising a zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a tau, APP, amyloid beta or presenilin polypeptide, which is expressed in the neurons of the transgenic zebrafish, where the transgenic zebrafish exhibits a pathology associated with Alzheimer's Disease, or (b) a tau, APP or presenilin fusion polypeptide, comprising a zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a fusion polypeptide comprising a tau, APP or presenilin polypeptide and a fluorescent reporter polypeptide, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: 1) a transgenic zebrafish that expresses a Tau (fusion) polypeptide; 2) a transgenic zebrafish that expresses an APP (fusion) polypeptide;3) a transgenic zebrafish that expresses an amyloid beta polypeptide;4) a transgenic zebrafish that expresses a presenilin (fusion) polypeptide;

BIOTECHNOLOGY - Preferred Zebrafish: The zebrafish further comprises zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a fluorescent reporter polypeptide e.g. green fluorescent protein (GFP), Aequorea coerulescens green fluorescent protein (AcGFP) and DsRedExpress (DsRed protein). The neuron specific expression sequence is a neuron-specific promoter chosen from an elav promoter and a GATA-2 promoter. The zebrafish neuron specific expression sequence and the sequence encoding the tau, APP, amyloid beta polypeptide are contained in an exogenous construct. The zebrafish develops neurofibrillary tangles, or exhibits neuronal cell damage. The tau, APP polypeptide, amyloid beta or presenilin is a mutant tau, APP, amyloid beta polypeptide or presenilin. The expression sequence comprises an inducible promoter, being an inducible UAS promoter activated by GAL4/VP16. The zebrafish further comprises a nucleic acid encoding a zinc transporter. Preferred Method: Identifying an agent that modulates a pathology associated with disease comprises: a) contacting the zebrafish with a test agent; b) comparing the neuronal pathology of the zebrafish contacted with the test agent to the neuronal pathology of a zebrafish not contacted with the test agent; c) determining the effect of the test agent on the zebrafish, such that if there is a difference in the neuronal pathology of the zebrafish contacted with the test agent and the zebrafish not contacted with the test agent, the test agent is an agent that modulates a pathology associated with Alzheimer's disease. The difference in neuronal pathology is a decrease in neuronal cell death in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent or a decrease in neurofibrillary tangles in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent. The difference is neuronal pathology is a decrease in neuronal fluorescence. The difference in neuronal pathology is a decrease in protein expression in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent. Identifying an agent that modulates neuronal pathology comprises: a) administering a test agent to a transgenic zebrafish expressing a reporter protein in neurons; b) comparing the expression of the reporter protein in the neurons of the zebrafish contacted with the test agent with the expression of the reporter protein in the neurons of a transgenic zebrafish that was not contacted with the test agent; and c) determining the effect of the test compound on the expression of the reporter protein in the neurons, such that if the number of neurons in the zebrafish contacted with the test agent is greater than the number of neurons in the zebrafish that was not contacted with the test agent, the test agent is an agent that modulates neuronal pathology and is a neuroproliferative agent. The reporter protein is a fluorescent reporter polypeptide.

ACTIVITY - Nootropic; Neuroprotective. No biological data given.

MECHANISM OF ACTION - None given.

USE - For identifying an agent that modulates a pathology associated with Alzheimer's disease (claimed).

ADVANTAGE - The transgenic zebrafish enables identification of an

agent that modulates a pathology associated with Alzheimer's disease. (75 pages)

L4 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2007:30318 BIOSIS
DOCUMENT NUMBER: PREV200700030032
TITLE: The 1.7 angstrom crystal structure of Dronpa: A photoswitchable green fluorescent protein.
AUTHOR(S): Wilmann, Pascal G.; Turcic, Kristina; Battad, Jion M.; Wilce, Matthew C. J.; Devenish, Rodney J.; Prescott, Mark [Reprint Author]; Rossjohn, Jamie
CORPORATE SOURCE: Monash Univ, Prot Crystallog Unit, Clayton, Vic 3800, Australia
Mark.Prescott@med.monash.edu.au; Jamie.Rossjohn@med.monash.edu.au
SOURCE: Journal of Molecular Biology, (NOV 24 2006) Vol. 364, No. 2, pp. 213-224.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Dec 2006
Last Updated on STN: 27 Dec 2006

AB The green fluorescent protein (GFP), its variants, and the closely related GFP-like proteins possess a wide variety of spectral properties that are of widespread interest as biological tools. One desirable spectral property termed photoswitching, involves the light-induced alteration of the optical properties of certain GFP members. Although the structural basis of both reversible and irreversible photoswitching events have begun to be unraveled, the mechanisms resulting in reversible photoswitching are less clear. A novel GFP-like protein, Dronpa, was identified to have remarkable light-induced photoswitching properties, maintaining an almost perfect reversible photochromic behavior with a high fluorescence to dark state ratio. We have crystallized and subsequently determined to 1.7 angstrom resolution the crystal structure of the fluorescent state of Dronpa. The chromophore was observed to be in its anionic form, adopting a cis co-planar conformation. Comparative structural analysis of non-photoactivatable and photoactivatable GFPs, together with site-directed mutagenesis of a position (Cys62) within the Dronpa chromophore, has provided a basis for understanding Dronpa photoactivation. Specifically, we propose a model of reversible photoactivation whereby irradiation with light leads to subtle conformational changes within and around the environment of the chromophore that promotes proton transfer along an intricate polar network. (c) 2006 Elsevier Ltd. All rights reserved.

L4 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:456583 BIOSIS
DOCUMENT NUMBER: PREV200600457279
TITLE: The kindling fluorescent protein: A transient photoswitchable marker.
AUTHOR(S): Henderson, J. Nathan [Reprint Author]; Remington, S. James
CORPORATE SOURCE: Univ Oregon, Dept Chem, Eugene, OR 97403 USA
jremington@uoxray.uoregon.edu
SOURCE: Physiology (Bethesda), (JUN 2006) Vol. 21, pp. 162-170.
ISSN: 1548-9213.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Sep 2006
Last Updated on STN: 13 Sep 2006

AB Passive fluorescent protein markers are indispensable for dynamic cellular imaging; however, they are unselective, introduce constant background flourescence, and require continuous observation. Photoactivatable

flourescent proteins have now been developed whose fluorescence can be switched on and off by illumination, allowing selective and direct tracking of tagged objects without the need for continuous imaging. The "kindling fluorescent protein" is a photoactivatable marker with a novel twist: it turns itself off after a selectable period.

L4 ANSWER 4 OF 7 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 1

ACCESSION NUMBER: 2006357051 EMBASE
TITLE: Practical three color live cell imaging by widefield microscopy.
AUTHOR: Xia J.; Kim S.H.H.; Macmilllan S.; Truant R.
CORPORATE SOURCE: R. Truant, Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, ON L8N 3Z5, Canada. truantr@mcmaster.ca
SOURCE: Biological Procedures Online, (21 Jul 2006) Vol. 8, No. 1, pp. 63-68. .
Refs: 23
ISSN: 1480-9222 E-ISSN: 1480-9222 CODEN: BLPOF8
COUNTRY: Canada
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 17 Aug 2006
Last Updated on STN: 17 Aug 2006

AB Live cell fluorescence microscopy using fluorescent protein tags derived from jellyfish and coral species has been a successful tool to image proteins and dynamics in many species. Multi-colored aequorea fluorescent protein (AFP) derivatives allow investigators to observe multiple proteins simultaneously, but overlapping spectral properties sometimes require the use of sophisticated and expensive microscopes. Here, we show that the aequorea coerulescens fluorescent protein derivative, PS-CFP2 has excellent practical properties as a blue fluorophore that are distinct from green or red fluorescent proteins and can be imaged with standard filter sets on a widefield microscope. We also find that by widefield illumination in live cells, that PS-CFP2 is very photostable. When fused to proteins that form concentrated puncta in either the cytoplasm or nucleus, PSCFP2 fusions do not artifactually interact with other AFP fusion proteins, even at very high levels of over-expression. PSCFP2 is therefore a good blue fluorophore for distinct three color imaging along with eGFP and mRFP using a relatively simple and inexpensive microscope. .COPYRGT. 2006 by the author(s).

L4 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2005:660610 HCAPLUS
DOCUMENT NUMBER: 143:130107
TITLE: Preparation of transgenic theaceous plants
INVENTOR(S): Tanba, Yasuo; Kato, Michiyo
PATENT ASSIGNEE(S): Hamamatsu Foundation for Science and Technology Promotion, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 16 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2005198600	A	20050728	JP 2004-9649	20040116
PRIORITY APPLN. INFO.:			JP 2004-9649	20040116

AB The fluorescent heterologous gene(s) is introduced specifically into organelles of the embryonic callus for easy and non-invasive selection of transgenic theaceous plants. The organelles are selected from mitochondria and plastid. The fluorescence is obtained from the (mutated) green fluorescence protein, especially that of Aequorea coerulescens. Transformation of tea tree using mitochondria- and plastid-specific binary vectors was shown. Also given was the construction of mitochondria- and plastid-specific binary vectors using 35Ω-sGFP plasmid containing CMV 35S promoter, sGFP (S65T) gene, nopaline synthase polyadenylation signal, etc.

L4 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:591209 HCAPLUS
DOCUMENT NUMBER: 139:129175
TITLE: Sequences of novel fluorescent proteins from Aequorea coerulscens and use
INVENTOR(S): Gurskaya, Nadejda; Fradlov, Arkadiy; Lukyanov, Sergey; Punkova, Natalia
PATENT ASSIGNEE(S): Evrogen, Jsc, USA
SOURCE: PCT Int. Appl., 76 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003062270	A2	20030731	WO 2003-IB907	20030117
WO 2003062270	A3	20031127		
WO 2003062270	B1	20040401		
WO 2003062270	A8	20041104		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2474108	A1	20030731	CA 2003-2474108	20030117
EP 1485481	A2	20041215	EP 2003-706812	20030117
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2005526495	T	20050908	JP 2003-562147	20030117
US 2006167225	A1	20060727	US 2004-501629	20040715
PRIORITY APPLN. INFO.:			US 2002-351518P P	20020122
			WO 2003-IB907 W	20030117

AB The present invention provides protein and cDNA sequences of a novel colorless GFP-like protein, acGFP, from Aequorea coerulscens and fluorescent and non-fluorescent mutants and derivs. thereof, as well as peptides and proteins encoded by these nucleic acid compns. The subject protein and nucleic acid compns. of the present invention are colored and/or fluorescent and/or can be photoactivated, and can be used in a variety of different biol. applications, particularly for labeling. Finally, kits for use in such biol. applications are provided.

L4 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003313870 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12693991
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa

Aequorea coerulescens and its fluorescent mutants.

AUTHOR: Gurskaya Nadya G; Fradkov Arkady F; Pounkova Natalia I; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Labas Yulii A; Lukyanov Sergey; Lukyanov Konstantin A

CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow 117997, Russia.

SOURCE: The Biochemical journal, (2003 Jul 15) Vol. 373, No. Pt 2, pp. 403-8.
Journal code: 2984726R. ISSN: 0264-6021.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AY151052; GENBANK-AY233272

ENTRY MONTH: 200308

ENTRY DATE: Entered STN: 8 Jul 2003
Last Updated on STN: 16 Aug 2003
Entered Medline: 15 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222)-->Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Re-introduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

=> s "acgfpl"
L5 5 "ACGFPL"

=> d 1-5 ibib ab

L5 ANSWER 1 OF 5 MEDLINE on STN

ACCESSION NUMBER: 2003313870 MEDLINE

DOCUMENT NUMBER: PubMed ID: 12693991

TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.

AUTHOR: Gurskaya Nadya G; Fradkov Arkady F; Pounkova Natalia I; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Labas Yulii A; Lukyanov Sergey; Lukyanov Konstantin A

CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow 117997, Russia.

SOURCE: The Biochemical journal, (2003 Jul 15) Vol. 373, No. Pt 2, pp. 403-8.
Journal code: 2984726R. ISSN: 0264-6021.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY151052; GENBANK-AY233272
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 8 Jul 2003
Last Updated on STN: 16 Aug 2003
Entered Medline: 15 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222)-->Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Re-introduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L5 ANSWER 2 OF 5 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2003300328 EMBASE
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.
AUTHOR: Gurskaya N.G.; Fradkov A.F.; Pounkova N.I.; Staroverov D.B.; Bulina M.E.; Yanushevich Y.G.; Labas Y.A.; Lukyanov S.; Lukyanov K.A.
CORPORATE SOURCE: K.A. Lukyanov, Shemyakin/Ovchinnikov Inst. B., Miklukho-Maklaya 16/10, Moscow 117997, Russian Federation. kluk@ibch.ru
SOURCE: Biochemical Journal, (15 Jul 2003) Vol. 373, No. 2, pp. 403-408. .
Refs: 25
ISSN: 0264-6021 CODEN: BIJOAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 14 Aug 2003
Last Updated on STN: 14 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222) → Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Reintroduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and β-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L5 ANSWER 3 OF 5 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:378716 BIOSIS
DOCUMENT NUMBER: PREV200300378716
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.
AUTHOR(S): Gurskaya, Nadya G.; Fradkov, Arkady F.; Pounkova, Natalia I.; Staroverov, Dmitry B.; Bulina, Maria E.; Yanushevich, Yurii G.; Labas, Yulii A.; Lukyanov, Sergey; Lukyanov, Konstantin A. [Reprint Author]
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow, 117997, Russia kluk@ibch.ru
SOURCE: Biochemical Journal, (15 July 2003) Vol. 373, No. 2, pp. 403-408. print.
ISSN: 0264-6021.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Aug 2003
Last Updated on STN: 20 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu222 fwdarw Gly (E222G) substitution, which removed the evolutionarily invariant Glu222. Reintroduction of Glu222 into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L5 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:667579 SCISEARCH
THE GENUINE ARTICLE: 705HR
TITLE: Colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants
AUTHOR: Gurskaya N G; Fradkov A F; Pounkova N I; Staroverov D B; Bulina M E; Yanushevich Y G; Labas Y A; Lukyanov S; Lukyanov K A (Reprint)
CORPORATE SOURCE: RAS, Shemyakin & Ovchinnikov Inst Bioorgan Chem, Miklukho Maklaya 16-10, Moscow 117997, Russia (Reprint); RAS, Shemyakin & Ovchinnikov Inst Bioorgan Chem, Moscow 117997, Russia; Evrogen JSC, Moscow 117997, Russia
COUNTRY OF AUTHOR: Russia
SOURCE: BIOCHEMICAL JOURNAL, (15 JUL 2003) Vol. 373, Part 2, pp. 403-408.
ISSN: 0264-6021.
PUBLISHER: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 25
ENTRY DATE: Entered STN: 22 Aug 2003
Last Updated on STN: 22 Aug 2003

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coeridescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222)) --> Gly (E-222G) substitution, which removed the evolutionarily invariant Glu(222). Reintroduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and P-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L5 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:655855 HCAPLUS
DOCUMENT NUMBER: 139:376885
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants
AUTHOR(S): Gurskaya, Nadya G.; Fradkov, Arkady F.; Pounkova, Natalia I.; Staroverov, Dmitry B.; Bulina, Maria E.; Yanushevich, Yurii G.; Labas, Yulii A.; Lukyanov, Sergey; Lukyanov, Konstantin A.
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, 117997, Russia
SOURCE: Biochemical Journal (2003), 373(2), 403-408
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have cloned an unusual colorless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu222 → Gly (E222G) substitution, which removed the evolutionarily invariant Glu222. Reintroduction of Glu222 into the most fluorescent random mutant, named aceGFP, converted it into a colorless protein. This colorless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biol. tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of "humanized" aceGFP and β-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007
L1 115659 S GREEN (W)FLUORESCENT

```
L2            22 S AEQUOREA (W) COERULESCENS
L3            13 S L1 AND L2
L4             7 DUP REM L3 (6 DUPLICATES REMOVED)
L5             5 S "ACGFPL"

=> s "E222G" and l1
L6           20 "E222G" AND L1

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7            12 DUP REM L6 (8 DUPLICATES REMOVED)

=> d 1-12 ibib ab

L7   ANSWER 1 OF 12  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
ACCESSION NUMBER:    2007:11856  BIOSIS
DOCUMENT NUMBER:     PREV200700019448
TITLE:               Fluorescent proteins.
AUTHOR(S):           Anonymous; Stubbs, Simon Lawrence John [Inventor]; Jones,
                     Anne Elizabeth [Inventor]; Michael, Nigel Paul [Inventor];
                     Thomas, Nicholas [Inventor]
CORPORATE SOURCE:    Amersham, United Kingdom
                     ASSIGNEE: GE Healthcare Limited
PATENT INFORMATION:  US 07091317 20060815
SOURCE:              Official Gazette of the United States Patent and Trademark
                     Office Patents, (AUG 15 2006)
                     CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE:       Patent
LANGUAGE:            English
ENTRY DATE:          Entered STN: 20 Dec 2006
                     Last Updated on STN: 20 Dec 2006
AB   The present invention provides novel engineered derivatives of
     green fluorescent protein (GFP) which have an amino acid
     sequence which is modified by amino acid substitution compared with the
     amino acid sequence of wild type Green Fluorescent
     Protein.  The modified GFPs exhibit enhanced fluorescence relative to
     wtGFP when expressed in non-homologous cells at temperatures above 30
     degrees C., and when excited at about 490 nm compared to the parent
     proteins, i.e. wtGFP.  An example of a preferred protein is F64L-S175G-
     E222G-GFP.  The modified GFPs provide a means for detecting GFP
     reporters in mammalian cells at lower levels of expression and/or
     increased sensitivity relative to wtGFP.  This greatly improves the
     usefulness of fluorescent proteins in studying cellular functions in
     living cells.

L7   ANSWER 2 OF 12  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
ACCESSION NUMBER:    2006:335529  BIOSIS
DOCUMENT NUMBER:     PREV200600339985
TITLE:               Fluorescent proteins.
AUTHOR(S):           Bjorn, Sara Petersen [Inventor]; Pagliaro, Len [Inventor];
                     Thastrup, Ole [Inventor]
CORPORATE SOURCE:    Lyngby, Denmark
                     ASSIGNEE: Bioimage A/S
PATENT INFORMATION:  US 07001986 20060221
SOURCE:              Official Gazette of the United States Patent and Trademark
                     Office Patents, (FEB 21 2006)
                     CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE:       Patent
LANGUAGE:            English
ENTRY DATE:          Entered STN: 5 Jul 2006
                     Last Updated on STN: 5 Jul 2006
AB   A GFP with an F64L mutation and an E222G mutation is provided.
     This GFP has a bigger Stokes shift compared to other GFPs making it very
     suitable for high throughput screening due to a better resolution.  This
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GFP also has an excitation maximum between the yellow GFP and the cyan GFP allowing for cleaner band separation when used together with those GFPs.

L7 ANSWER 3 OF 12 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:243456 BIOSIS
DOCUMENT NUMBER: PREV200600252003
TITLE: Fluorescent proteins.
AUTHOR(S): Stubbs, Simon Lawrence John [Inventor]; Jones, Anne Elizabeth [Inventor]; Michael, Nigel Paul [Inventor]; Thomas, Nicholas [Inventor]
CORPORATE SOURCE: Amersham, United Kingdom
ASSIGNEE: Amersham plc
PATENT INFORMATION: US 06919186 20050719
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (JUL 19 2005)
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Apr 2006
Last Updated on STN: 26 Apr 2006
AB The present invention provides novel engineered derivatives of green fluorescent protein (GFP) which have an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein. The modified GFPs exhibit enhanced fluorescence relative to wtGFP when expressed in non-homologous cells at temperatures above 30 degrees C., and when excited at about 490 nm compared to the parent proteins, i.e. wtGFP. An example of a preferred protein is F64L-S175G-E222G-GFP. The modified GFPs provide a means for detecting GFP reporters in mammalian cells at lower levels of expression and/or increased sensitivity relative to wtGFP. This greatly improves the usefulness of fluorescent proteins in studying cellular functions in living cells.

L7 ANSWER 4 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-22091 BIOTECHDS
TITLE: New cytochrome c-reporter fusion protein construct comprising a modified cytochrome c protein, useful for detecting apoptosis in a cell;
involving vector-mediated gene transfer and expression in HEK-293
AUTHOR: STUBBS S L J; FRANCIS M J; CUSHING A; ISMAIL R A
PATENT ASSIGNEE: AMERSHAM BIOSCIENCES UK LTD
PATENT INFO: WO 2005058960 30 Jun 2005
APPLICATION INFO: WO 2004-GB5317 17 Dec 2004
PRIORITY INFO: GB 2003-29353 19 Dec 2003; GB 2003-29353 19 Dec 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-506111 [51]
AB DERWENT ABSTRACT:
NOVELTY - A cytochrome c-reporter fusion protein construct comprising a modified cytochrome c protein or any of its functional analogue derived from wild type cytochrome c, where the modified cytochrome c targets the mitochondria and has a reduced ability to induce apoptosis in a living cell, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a nucleotide sequence encoding a fusion construct above; (2) a nucleic acid construct comprising a control region and a nucleotide sequence of (1), the sequence being under the control of the control region; (3) a replicable vector comprising a nucleic acid construct of (2); (4) a host cell stably or transiently transformed with a nucleic acid construct of (2); (5) detecting apoptosis in a living cell; and (6) measuring the effect that an agent has upon modulating apoptosis in a living cell.
BIOTECHNOLOGY - Preferred Fusion Construct: The modified cytochrome

c binds apoptosis protease activation factor-1 (Apaf-1) at least 10-1000 times less than wild type cytochrome c. At least one of the amino acids of the modified cytochrome c at positions 4, 7, 8, 25, 39, 62, 63, 64, 65 and 72 has been mutated relative to the wild type cytochrome c, where the modified cytochrome c has an amino substitution or substitutions selected from K4E, K72A, K72L, K72R, K72G, K72X, E62N, K7E-K8E, K25P-K39H, K7A-E62N-K25P, K7A-E62N-K39H, K7E-K8E-E62N, K7A-K25P-E62N, K7A-E62N-K25P-K39H, E62N-T63N-L64M-M65S, preferably K7E-K8E-E62N-K25P-K39H, K7E-K8E-K25P-E62N-T63N-L64M-M65S, K7E-K8E-K39H-E62NT63N-L64M-M65S or K7E-K8E-K25P-K39H-E62N-T63N-L64M-M65S. The modified cytochrome c comprises the amino acid substitution selected from K72A, K72L, K72R, K72G or K72X, where X represents trimethylation, preferably the modified cytochrome c comprises the amino acid substitution K72A or K72L. The modified cytochrome c comprises the amino acid substitution K4E. The reporter is a fluorescent protein or its functional analogue, where the fluorescent protein is Green Fluorescent Protein (GFP), Yellow Fluorescent Protein (YFP), Blue Fluorescent Protein (BFP), Cyan Fluorescent Protein (CFP), Red Fluorescent Protein (RFP), preferably Enhanced Green Fluorescent Protein (EGFP) or Emerald, where the GFP comprises an amino acid substitution at position F64L, S175G, or E222G. The fusion construct comprises a sequence of 348 or 347 amino acids (SEQ ID NOS: 4 or 6). The reporter is localizable by a detectable luminescent, fluorescent or radioactive moiety, where the reporter comprises an immunogenic motif or a cysteine-rich motif, and where the detectable moiety comprises a bi-arsenical compound or an antibody. Preferred Nucleic Acid Sequence: The nucleotide sequence encoding the fusion construct comprises a sequence of 1044 or 1041 bp (SEQ ID NOS: 3 or 5). Preferred Nucleic Acid Construct: The promoter is native cytochrome c promoter, mammalian constitutive promoter, mammalian regulatory promoter, human ubiquitin C promoter, viral promoter, yeast promoter, filamentous fungal promoter, or bacterial promoter, where the viral promoter is the CMV or the SV40 promoter. The promoter is the human ubiquitin C promoter. Preferred Replicable Vector: The vector is a plasmid vector or a viral vector selected from cytomegalovirus, Herpes simplex virus, Epstein-Barr virus, Simian virus 40, Bovine papillomavirus, Adeno-associated virus, Adenovirus, Vaccina virus or Baculovirus vector. Preferred Host Cell: The host cell is a plant, insect, nematode, bird, fish and mammalian cell, where the mammalian cell is a human cell. The human cell is Hek, HeLa, U2OS or MCF-7, where the Hek cell is Hek293. The host cell is capable of expressing the fusion protein described above. Preferred Method: Detecting apoptosis in a living cell comprises culturing a cell transformed to over-express a fusion construct described above and determining the localization of the fusion construct within the cell with time, where a change in localization of the fusion construct within the cell is indicative of apoptosis. Measuring the effect that an agent has upon modulating apoptosis in a living cell comprises culturing a cell transformed to over-express a fusion construct described above, determining the localization of the construct within the cell, and treating the cell with the agent and determining the localization of the construct within the cell, where any difference in the localization of the construct within the cell relative to control cells untreated with the agent is indicative of the effect that the agent has upon modulating apoptosis. Alternatively, the method comprises culturing a first cell and a second cell which both over-express a fusion construct described above, treating the first cell with the agent and determining the localization of the construct within the first cell, and determining the localization of the construct within the second cell which has not been treated with the agent, where any difference in the localization of the construct within the first cell and second cell is indicative of the effect that the agent has upon modulating apoptosis. Measuring the effect an agent has upon modulating apoptosis in a living cell may also comprise culturing a cell transformed to over-express a fusion construct described above, treating the cell with the agent and determining the localization of the construct

within the cell, and comparing the localization of the construct in the presence of the agent with a known value for the localization of the construct in the absence of the agent, where any difference in the localization of the construct within the cell in the presence of the agent and the known value in the absence of the agent is indicative of the effect that the agent has upon modulating apoptosis. The known value is stored on a database. The localization of the fusion construct is measured by its luminescence, fluorescence or radioactive properties. The agent induces or inhibits apoptosis. The localization of the protein fusion is determined following fixation of the cells. The agent is a chemical, physical or biological agent.

USE - The fusion protein construct, nucleic acid construct, and methods are useful for detecting apoptosis in a cell.(56 pages)

L7 ANSWER 5 OF 12 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003313870 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12693991
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.
AUTHOR: Gurskaya Nadya G; Fradkov Arkady F; Pounkova Natalia I; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Labas Yulii A; Lukyanov Sergey; Lukyanov Konstantin A
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow 117997, Russia.
SOURCE: The Biochemical journal, (2003 Jul 15) Vol. 373, No. Pt 2, pp. 403-8.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY151052; GENBANK-AY233272
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 8 Jul 2003
Last Updated on STN: 16 Aug 2003
Entered Medline: 15 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222)-->Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Re-introduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L7 ANSWER 6 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-06533 BIOTECHDS
TITLE: Novel fluorescent protein derived from green fluorescent protein useful as a transfection marker, has different excitation spectrum and/or emission spectrum compared with wild-type green fluorescent protein;

recombinant green fluorescent protein production in transformed mammal, bacterium, yeast or insect cell culture

AUTHOR: STUBBS S L J; JONES A E; MICHAEL N P; THOMAS N
PATENT ASSIGNEE: AMERSHAM PHARMACIA BIOTECH UK LTD; AMERSHAM BIOSCIENCES UK LTD
PATENT INFO: GB 2374868 30 Oct 2002
APPLICATION INFO: GB 2001-23288 28 Sep 2001
PRIORITY INFO: GB 2001-9858 23 Apr 2001; GB 2001-9858 23 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-095652 [09]

AB DERWENT ABSTRACT:
NOVELTY - A fluorescent protein (I) derived from green fluorescent protein (GFP) or any functional GFP analog, has an amino acid sequence which is modified by amino acid substitution at position F64, at position S65 or E222, and at position S175 compared with the amino acid sequence of wild-type GFP, and has different excitation spectrum and/or emission spectrum compared with wild-type GFP, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fusion compound (II) comprising a protein of interest fused to (I); (2) a nucleic acid molecule (III) comprising a nucleotide sequence encoding (I) or (II); (3) an expression vector (IV) comprising suitable expression control sequences operably linked to (III); and (4) a host cell (V) transformed or transfected with a DNA construct comprising (IV).

BIOTECHNOLOGY - Preparation: (I) is prepared by cultivating (V) and obtaining the polypeptide expressed by the nucleotide sequence (claimed). Preferred Protein: In (I), the amino acid F at position 64 is substituted by an amino acid L, I, V, A or G. The amino acid S at position 175 is substituted by an amino acid G, A, L, I or T. The amino acid S at position 65 is substituted by an amino acid G, A, L, C, V, I or T. The amino acid E at position 222 is substituted by an amino acid G, A, V, L, I, F, S, T, N or Q. (I) is F64L-S175G-E222G-GFP or F64L-S65T-S175G-GFP. (I) has an amino acid sequence which is modified by amino acid substitution compared with wild-type GFP having a sequence of 238 amino acids fully defined in the specification. Preferred Host Cell: (V) is a mammalian cell, bacterial cell, yeast cell, or an insect cell.

USE - (III) is useful for measuring the expression of a protein of interest in a cell, by introducing (III) into a cell, where (III) is operably linked to and under the control of an expression control sequence which moderates expression of the protein of interest, culturing the cell under conditions suitable for the expression of the protein of interest, and detecting the fluorescence emission of GFP or functional GFP analog. (III) is useful for determining the cellular and/or extracellular localization of a protein of interest. (III) is also useful for comparing the effect of one or more test substance(s) on the expression and/or localization of one or more different protein(s) of interest in a cell. The method involves: (a) introducing into a cell, (III) operably linked to and under the control of a first expression control sequence and optionally fused to a nucleotide sequence encoding a fusion protein of interest, and optionally, at least one different nucleic acid molecule encoding a protein reporter molecule fused to a different protein of interest, where the nucleic acid molecule is operably linked to and under the control of a second expression control sequence, and the protein reporter molecule has or is capable of generating an emission signal which is spectrally distinct from that of GFP or functional GFP analog; (b) culturing the cells under conditions suitable for the expression of the protein(s) of interest in the presence and absence of the test substance(s); (c) determining the expression and/or localization of the protein(s) in the cells by detecting the fluorescence emission by optical means; and (d) comparing the fluorescence emission obtained in the presence and absence of the test substance(s). The samples of the cells in a fluid medium are introduced

into separate vessels for each of the test substances to be studied (all claimed). (I) is useful as a non-toxic marker for selection of transfected cells, as a protein label in living and fixed cells, as a marker in cell or organelle fusion, for visualizing translocation of intracellular proteins to a specific organelle, as a secretion marker, as genetic reporter or protein tag for protein and gene expression in transgenic animals, as a cell or organelle integrity marker, as a transfection marker, as a marker to be used in combination with fluorescent activated cell sorting (FACS), as real-time probe working at near physiological concentrations, for performing transposon vector mutagenesis, and as a reporter for bacterial detection.

ADVANTAGE - (I) exhibits enhanced fluorescence relative to wild type GFP, when expressed in non-homologous cells at temperatures above 30degreesC, and excited at 490 nm. (I) detects GFP reporters in mammalian cells at lower levels of expression with increased sensitivity relative to wild type GFP.

EXAMPLE - Generation of mutants of green fluorescent protein (GFP) was as follows. The GFP gene was contained within the plasmid pGFP. The gene was amplified by polymerase chain reaction (PCR) using plaque forming units (pfu) polymerase. The primers were GFP-1: 5'-ggtacgggccgccaccatgagtaaaggagaagaactttcac, GFP-2: 5'-ggtacgggttaaccggttttgtatagttcatccatg, and GFP-3: 5'-ggtacgggccgccaccatgggatccaaaggagaagaacttttcac. Amplified products resulting from PCR reactions were tailed with a single 3'-deoxyadenosine using Taq polymerase and ligated into the TA cloning vector pTARGET. The mutants of GFP gene (encoding a sequence of 238 amino acids fully defined in the specification) construct such as F64L-S175G-E222G-GFP and F64L-S65T-S175-GFP within pTARGET were generated using the QuickChange site-directed mutagenesis kit. The primers used for F64L were GFP-64f: ccaacacttgtcactactctctcttatggtgttcaat and GFP-64r: attgaacaccataagagagagtagtgacaagtgttgg, S65T were GFP-65f: ccaacacttgtcactactctcacctatggtgttcaatgcttttca and GFP-65r: tgaaaagcattgaacaccataggtgagagtagtgacaagtgttgg, S175G were GFP-175f: caacatgaagatggaggcgttcaactagcagacc and GFP-175r: ggtctgctagttgaacgcctccatcttcaatgttg, and E222G were (GFP-222f: ccacatggtccttcttggctttgtaacagctgctgg and GFP-222r: ccagcagctgttacaaagccaagaaggaccatgtgg). Multiply-mutated GFP molecules were generated through successive mutagenesis reactions. All GFP mutant sequences were verified by automated sequencing. The influence of individual mutations and combinations of F64L, S65T, V163A, S175G and E222G mutations upon GFP when expressed in mammalian cells was evaluated. Plasmid DNA to be used for transfection was prepared. DNA was diluted to 100 ng.microl-1 in 18-Megohm water and 1 microg used for transfections. For 50-80% confluency on the day of transfection, HeLa cells were plated at a density of 5x104/well in 6-well plates and incubated overnight. A 1:3 (1 microg:3 microl) ratio of DNA to FuGene6 reagent was used for each transient transfection reaction. 3microl FuGene 6 was added to 87 microl serum-free Dulbecco's modified Eagle medium (DMEM) and gently tapped to mix. Then 10 microl (1 microg) construct DNA was added and again gently mixed. The FuGene6:DNA complex was incubated at room temperature for 40 minutes, then added dropwise directly to the cells without changing the medium, and the plates swirled for even distribution. Fluorescence measurements were made 24 and 48 h after transfection. Average fluorescent intensities from fluorescent activated cell sorting (FACS) analysis were obtained. (52 pages)

L7 ANSWER 7 OF 12 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2002:832828 HCAPLUS

DOCUMENT NUMBER: 137:334476

TITLE: Preparation of green fluorescent protein mutants with enhanced fluorescence for use as reporter proteins

INVENTOR(S): Stubbs, Simon Lawrence John; Jones, Anne Elizabeth; Michael, Nigel Paul; Thomas, Nicholas

PATENT ASSIGNEE(S): Amersham Biosciences UK Ltd., UK
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002085936	A1	20021031	WO 2001-GB4363	20010928
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
GB 2374868	A	20021030	GB 2001-23288	20010928
GB 2374868	B	20030709		
CA 2445035	A1	20021031	CA 2001-2445035	20010928
AU 2001292040	A1	20021105	AU 2001-292040	20010928
US 2003175859	A1	20030918	US 2001-967301	20010928
US 6919186	B2	20050719		
EP 1381625	A1	20040121	EP 2001-972260	20010928
EP 1381625	B1	20041124		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
AT 283283	T	20041215	AT 2001-972260	20010928
JP 2005502323	T	20050127	JP 2002-583462	20010928
ES 2233690	T3	20050616	ES 2001-1972260	20010928
US 2004138420	A1	20040715	US 2004-757624	20040114
US 7091317	B2	20060815		
US 2006036078	A1	20060216	US 2005-251209	20051014

PRIORITY APPLN. INFO.:		
GB 2001-9858	A	20010423
US 2001-967301	A3	20010928
WO 2001-GB4363	W	20010928
US 2004-757624	A3	20040114

AB The present invention provides novel engineered derivs. of green fluorescent protein (GFP) which have an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein. The modified GFPs exhibit enhanced fluorescence relative to wtGFP when expressed in non-homologous cells at temps. above 30 °C, and when excited at about 490 nm compared to the parent proteins, i.e. wtGFP. An example of a preferred protein is F64L-S175G-E222G-GFP. The modified GFPs provide a means for detecting GFP reporters in mammalian cells at lower levels of expression and/or increased sensitivity relative to wtGFP. This greatly improves the usefulness of fluorescent proteins in studying cellular functions in living cells.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 12 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:31756 HCAPLUS
DOCUMENT NUMBER: 136:82316
TITLE: A method for extracting quantitative information relating to interactions between cellular components
INVENTOR(S): Terry, Bernard Robert; Hagel, Grith; Thastrup, Ole; Bjorn, Sara Petersen
PATENT ASSIGNEE(S): Bioimage A/s, Den.
SOURCE: PCT Int. Appl., 89 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002003072	A2	20020110	WO 2001-DK466	20010703
WO 2002003072	A3	20020613		
WO 2002003072	A8	20020919		
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2414626	A1	20020110	CA 2001-2414626	20010703
EP 1301796	A2	20030416	EP 2001-949277	20010703
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2004502434	T	20040129	JP 2002-508083	20010703
US 2004018504	A1	20040129	US 2003-332065	20030314

PRIORITY APPLN. INFO.:

DK 2000-1041	A	20000704
DK 2001-775	A	20010516
WO 2000-PA1041	A	20000704
WO 2001-PA775	A	20010516
WO 2001-DK466	W	20010703

AB A method is described to assay for protein interactions in living cells. The method uses the measurement of cellular distribution of a detectable component to indicate the presence or absence of an interaction between that component and a second component of interest. The method uses the knowledge that certain components can be stimulated to redistribute within the cell to defined locations. Inducible redistribution systems make it possible to determine if specific interactions occur between components. Inducible systems are described where it is demonstrated that the redistribution stimuli are essentially "null", in that they affect no other system in the cell during the assay period, other than the component whose redistribution can be induced. Also described is an extraction buffer which is useful in high throughput screening for drugs which affect the intracellular distribution of intracellular components. The extraction buffer comprises a cellular fixation agent and cellular permeabilization agent. Optimizing the composition of the extraction buffer and its application to various cell types is described.

L7 ANSWER 9 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2002-07557 BIOTECHDS
TITLE: Novel fluorescent protein in in vitro assay for measuring protein kinase activity or dephosphorylation activity, or for measuring protein redistribution, has a green fluorescent protein with F64L and E222G mutation;
recombinant protein production via plasmid expression in bacterium
AUTHOR: BJORN S P; PAGLIARO L; THASTRUP O
PATENT ASSIGNEE: BIOIMAGE AS
PATENT INFO: WO 2001098338 27 Dec 2001
APPLICATION INFO: WO 2000-EP6848 19 Jun 2000

PRIORITY INFO: US 2001-290170 10 May 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-098224 [13]

AB DERWENT ABSTRACT:

NOVELTY - A fluorescent protein (I), F64L, E222G-GFP derived from green fluorescent protein (GFP) or its analog comprising a mutated amino acid at position 1, which lies before a chromophore and a mutation at position 222 having a glutamic acid, and when expressed in cells incubated at 30 degrees C, has an excitation maximum at a higher wavelength and increased fluorescence, compared to wild-type GFP, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fusion compound (II) consisting of (I) linked to a polypeptide;(2) a nucleotide sequence (III) coding for (I); (3) a host cell (IV) transformed with (III); and (4) preparation of (I), comprising culturing (IV) under expression conditions, and recovering the polypeptide.

BIOTECHNOLOGY - Preferred Protein: The chromophore is in position 65-67 of the predicted primary amino acid sequence of GFP, where preferably amino acid F in position 64 is substituted by an aliphatic amino acid which is Leu, Ile, Val, Ala or Gly, and glutamic acid in position 222 is substituted by amino acid from Gly, Ala, Val, Leu, Ile, Phe, Ser, Thr, Asn, and Gln. The protein is derived from Aequoria victorea or Renilla. Preferred Fusion Compound: The polypeptide of (II) is a kinase which is the catalytic subunit of protein kinase A, protein kinase C, Erk1, or a cytoskeletal element.

USE - (I) is useful in an in vitro assay for measuring protein kinase activity or dephosphorylation activity, or for measuring protein redistribution (claimed). (I) is useful in studying cellular functions in living cells; as a protein tag in living and fixed cells, organelle tag, a secretion marker, genetic reporter or as protein tag in transgenic animals. (I) is also useful as a cell or organelle integrity marker, a marker for changes in cell morphology, as transfection marker and as a marker to be used in combination with fluorescence activated cell sorting (FACS), as real-time probe working at near physiological concentration. The novel proteins can also be used as reporters to monitor live/dead biomass of organisms, such as fungi. (I) is useful as markers useful in transcriptional and translational fusions for performing transposon vector mutagenesis. Transposons encoding (I) are useful for screening promoters, and transposon vectors encoding (I) can be used for tagging plasmids and chromosomes. (I) is useful as a reporter for bacterial detection, by introducing (I) into the genome of bacteriophages. Further, by engineering (I) into genome of a phage, is useful for designing diagnostic tool.

ADVANTAGE - The increased stokes shift of F64L, E222G-GFP results in increased spectral resolution of its excitation and emission peaks. This enables more complete band separation using a conventional dichroic beam-splitter, and decreased background signal for assays incorporating F64L, E222G-GFP relative to assays based on EGFP (F64L, S65T-GFP). F64L, E222G-GFP fluorescence can be excited by conventional light source using narrow band filters, or commercially available laser producing lines at 472 nm. In either case, the greater stokes shift of F64L, E222G-GFP results in lower cross-talk from excitation light to the toe of the emission spectrum. The excitation maximum of F64L, E222G-GFP falls midway between those of the cyan fluorescent protein variant (ECFP, excitation max about 433 nm) and the yellow fluorescent protein variant (EYFP, excitation max about 513 nm). Because of this, it will allow for cleaner band separation when used together with those probes, and it is optimized for assay applications in which several GFP-labeled components will be multiplexed. Due to strong fluorescence the novel proteins are suitable tags for proteins present at low concentrations. Since no substrate is needed and visualization of the cells does not damage the cells dynamic analysis can be performed. More

than one organelle can be tagged and visualized simultaneously in living cells, e.g. the endoplasmic reticulum and the cytoskeleton. By fusion of F64L-E222G-GFP to a signal peptide or a peptide to be secreted, secretion may be followed on-line in living cells. A precondition for that is that the maturation of a detectable number of novel fluorescent protein molecules occurs faster than the secretion. Due to strong fluorescence of the novel proteins, they are suitable as tags for proteins and gene expression, since the signal to noise ratio is significantly improved compared to the prior art proteins such as wild-type GFP. By co-expressing two of the novel proteins, the one targeted to an organelle and the other expressed in the cytosol, it is possible to calculate the relative leakage of the cytosolic protein and use that as a measure of cell integrity, and expression of the novel proteins in cells allows easy detection of changes in cell morphology, e.g. blebbing, caused by cytotoxic agents or apoptosis. The morphological changes are difficult to visualize in intact cells without the use of fluorescent probes. Due to the increased brightness of the novel proteins the quality of the cell detection and sorting can be significantly improved. Since F64L-E222G-GFP is significantly brighter than wild-type GFP and F64L-GFP when expressed in cells at 37 degrees C and excited with light at about 490 nm, the concentration needed for visualization can be lowered. Target sites for enzymes engineered into the novel proteins e.g. F64L-E222G-GFP, can therefore be present in the cell at low concentrations in living cells. This is important for the two reasons, i.e. the probe must interfere as little as possible with the intracellular process being studied; the translational and transcriptional apparatus should be stressed minimally. By constitutive expression of F64L-E222G-GFP in fungi the viable biomass will light up.

EXAMPLE - pEGFP-N1 and pEGFP-C1 were first subjected to polymerase chain reaction (PCR) with primers 9859 (5'-TGTACTAGTGACCACCCTGTCTTACGGCGT GCA-3') and 9860 (5'-CTGACTAGTGTGGGCCAGGGCACGGGCAGC-3') where the primers were complementary to the DNA sequence around the chromophore region and introduce a point mutation changing the threonine at position 65 to serine. In addition the primers introduce a unique Spe1 restriction site by silent mutation. The 4.7 kbase PCR products were digested with Spe1, religated, and transformed into Escherichia coli. The resulting plasmids were referred to as PS399 (N1 context) and PS401 (C1 context). These plasmids contained the chromophore sequence 64-LSY-67. Plasmids PS399 and PS401 were subjected to Quick-change mutagenesis (Stratagene) employing PCR with primers 0225 (5'-CCCGGCGGCGGTCACGAACCCTAGGAGGACCATGTGATCGCG-3') and 0226 (5'-CGCGATCACATGGTCCTCCTAGGGTTCGTGACCGCCGCCGGG-3'). These primers were complementary to sequences near the C-terminus of the green fluorescent protein (GFP) and change glutamate at position 222 to glycine, and in addition they introduce an Avr2 restriction site by silent mutation. The resulting plasmids were referred to as PS609 (N1 context) and PS701 (C1 context). They combine an LSYG chromophore with E222G with humanized codon and were referred to as eF64L, E222G. Plasmids expressing EGFP from plasmid pEGFP-N1 and eF664L, E222G from plasmid Ps699 were transfected into E. coli TOP10 cells (introgen) using lipofectamine 2000. After 5 days cells were collected and resuspended in extraction buffer 50 mM TRIS (pH8.0) with 1 mM dithiothreitol (DTT). Cells were lysed, and cells debris was centrifuged. The cell pellets were resuspended in 1000 micro-l of water each (2-fold dilution relative to volumes of pelleted cultures) and transferred to 1.0x0.5 cm plastic cuvettes and the following excitation and emission spectra were recorded on a Perkin Elmer LS50B luminescence spectrometer (excitation at 350-525 nm (5 nm slit width) and 430 nm (10 nm slit width). Using the same settings, excitation and emission spectra of 10-fold (200 micro-l of 2-fold diluted cells mixed with 800 micro-l of water) diluted cells were recorded for the strongly fluorescent samples expressed from cDNAs with jellyfish backbone (PS1185 and PS1186). The spectra recorded for the probes with Ser65:G222 (PS1186 and PS1191) were very similar (excitation and emission maxima at 468-473

nm and 505-506 nm, respectively) and with stokes shifts of 33-37 nm.(41 pages)

L7 ANSWER 10 OF 12 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-06807 BIOTECHDS
TITLE: New long wavelength engineered fluorescent proteins, useful as markers for gene expression, tracers of cell lineage or as fusion tags to monitor protein localization, or in detection assays, e.g. immunoassays or hybridization assays; genetically engineered protein, vector expression in host cell and DNA probe useful for designing mutant strain with altered anion binding and gene expression marker
AUTHOR: WACHTER R; REMINGTON S J
PATENT ASSIGNEE: UNIV OREGON STATE
PATENT INFO: WO 2001090147 29 Nov 2001
APPLICATION INFO: WO 2000-US16149 19 May 2000
PRIORITY INFO: US 2000-575847 19 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-083084 [11]
AB DERWENT ABSTRACT:
NOVELTY - A functional engineered fluorescent protein (I), whose is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (A-GFP) having a 238 residue amino acid sequence (I-a), fully defined in the specification, is new.

DETAILED DESCRIPTION - A functional engineered fluorescent protein (I), whose is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (A-GFP) having a 238 residue amino acid sequence (I-a), fully defined in the specification, is new. (I) has an amino acid sequence that: (a) differs from (I-a) by at least the amino acid substitution T203X; or (b) differs from (I-a) by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G) or V224. (I) has a different fluorescent property than A-GFP. X = an aromatic amino acid selected from H, Y, W or F. INDEPENDENT CLAIMS are also included for the following: (1) nucleic acid molecules comprising a nucleotide sequence encoding (I); (2) expression vectors comprising expression control sequences operatively linked to the nucleic acid molecule comprising a sequence encoding (I); (3) host cells comprising: (a) recombinant host cells comprising the expression vectors; or (b) (I) whose amino acid sequence differs from (I-a) by at least one first substitution at position T203, and at least one second substitution at position H148; (4) fluorescently labeled antibodies comprising antibodies coupled to (I); (5) nucleic acid molecules comprising nucleotide sequences encoding the antibodies fused to nucleotide sequences encoding (I); (6) fluorescently labeled nucleic acid probes comprising a nucleic acid probes coupled to (I); (7) determining if a mixture contains a target comprising: (a) contacting the mixture with the fluorescently labeled probes; and(b) determining if the target has bound to the probe; (8) engineering (I), which has a fluorescent property different from A-GFP; (9) producing fluorescence resonance energy transfers; (10) protein comprising (I), where the crystal diffracts with at least a 2.0-3.0 Angstrom resolution; (11) a computational method of designing a fluorescent protein; (12) a computational method of modeling the three dimensional structure of a fluorescent protein by determining a three dimensional relationship between at least two atoms listed in the atomic coordinates fully described in the specification; (13) a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from those atomic coordinates fully listed in the specification; (14) identifying a test chemical, comprising: (a) contacting a test chemical with a sample containing a biological entity labeled with (I) or a polynucleotide encoding (I); and (b) detecting fluorescence of (I); (15) determining the presence of an anion of interest in a sample, comprising: (a) introducing (I) into a sample; and

(b) determining the fluorescence of (I); and (16) screening the effects of test compounds on ion channel activity.

BIOTECHNOLOGY - Preferred Nucleic Acid: The amino acid sequence further comprises a mutation fully described in the specification, e.g. Y145F, V163A, N146I, M153T, V163A, N212K, I123V, Y145H, H148R, M153T, S65G, S72A, K79R or T203Y. The nucleic acid sequence encoding the protein differs from a 717 base pair sequence, fully defined in the specification by the substitution of at least one codon by a preferred mammalian codon. The nucleic acid molecule encodes a fusion protein, which comprises a polypeptide of interest and (I). Preferred Host Cell: The recombinant host cell is a prokaryotic cell or a eukaryotic cell. Preferred Protein: The amino acid sequence further comprises a substitution at S65, where the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. The amino acid sequence differs by no more than the substitutions S65T/T203H, S65T/T203Y, S72A/F64L/S65G/T203Y, S72A/S65G/V68L/T203Y, S65G/V68L/Q69K/S72A/T203Y, S65G/S72A/T203Y or S65G/S72A/T203W. The amino acid sequence further comprises a substitution at Y66, where the substitution is selected from Y66H, Y66F and Y66W. The amino acid sequence further comprises a folding mutation. The fusion protein comprises a polypeptide of interest and (I). Preferably, the fusion protein comprises the antibody fused to (I). In particular, the amino acid substitution comprises: (a) L42X, X is C, F, H, W or Y; (b) V6IX, X is F, Y, H or C; (c) T62X, X is A, V, F, S, D, N, Q, Y, H or C; (d) V68X, X is F, Y or H; (e) Q69X, X is K, R, E or G; (f) Q94X, X is D, E, H, K or N; (g) N121X, X is F, H, W or Y; (h) Y145X, X is W, C, F, L, E, H, K or Q; (i) H148X, X is F, Y, N, K, Q or R; (j) V150X, X is F, Y or H; (k) F165X, X is H, Q, W or Y; (l) I167X, X is F, Y or H; (m) Q183X, X is H, Y, E or K; (n) N185X, X is D, E, H, K or Q; (o) L220X, X is H, N, Q or T; (p) E222X, X is N and Q; or (q) V224X, X is H, N, Q, T, F, W or Y. At least an amino acid substitution is located no more than 0.5 nm from the chromophore of the engineered fluorescent protein, where the substitution alters the electronic environment of the chromophore, and where (I) has a different fluorescent property than A-GFP. The fluorescent protein, which comprises the crystal, has at least 200 amino acids, a completeness value of at least 80 % and has a crystal stability within 0.5 % of its unit cell dimensions. In particular, the crystal has the following unit cell dimensions in angstroms: a = 51.8, b = 62.8 and c = 70.7, with a space group of P2 2 2 and an alpha angle of 90.00 degrees, a beta angle of 90.00 degrees and a gamma angle of 90.00 degrees. The crystal has a diffraction limit, where 90 % or greater of the potential reflections can be used to determine the coordinates of the atoms. The substitution at Q69 is selected from K, R, E and G, where the amino acid sequence further comprises a function mutation at S65. The substitution at E222 is selected from N and Q, where the amino acid sequence further comprises a function mutation at F64. The substitution at Y145 is selected from W, C, F, L, E, H, K and Q, where the amino acid sequence further comprises a function mutation at Y66. The nucleotide sequence encodes a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of A-GFP and which differs from (I-a) by: (a) at least one first substitution at position T203, where the substitution consists of H, Y, W or F; and (b) at least one second substitution at position H148, where (I) has a different fluorescent property than Aequorea green fluorescent protein. At least one second substitution at position H148 is selected from H148R, H148G, H148Q, H148A, H148N and H148K. At least one second substitution at position H148 is H148Q. At least one second substitution at position H148 is H148G. At least one second substitution at position H148 is H148R. (I) further comprises at least one third substitution at position V150, where the substitution at position V150 consists of A, C, M, G, L, Q, S, T and N. (I) further comprises at least one third substitution at position V163, where the at least one third substitution at position V163 is selected from A, C, M, G, L, Q, S, T and N. (I) further comprises at least one-third substitution at position Q69, which consist of N, S, T and V. (I) may further comprise at least one-third substitution at

position 'V152, which comprises A, C, M, G, L, V, F, S, T, Q or N. (I) also comprises at least one third substitution at position F165, which consists of Y, L and W. (I) further comprises at least one third substitution at position H181, which consists of K, R, F, Y and W. (I) further comprises at least one third substitution at position L201, where the substitution is selected from A, C, M, G, S, T ,Q, N, V and I. Preferred Method: In method (7), the target is bound to a solid matrix. In method (8), engineering a functional engineered fluorescent protein having a fluorescent property different than A-GFP comprises substituting an amino acid that is located no more than 0.5 nm from any atom in the chromophore of an Aequorea-related GFP with another amino acid; where the substitution alters a fluorescent property of the protein. The amino acid substitution alters the electronic environment of the chromophore. Engineering (I) may also comprise substituting amino acids in a loop domain of an Aequorea-related GFP with amino acids to create a consensus sequence for phosphorylation or for proteolysis. In method (9), producing fluorescence resonance energy transfer comprises: (a) providing a donor molecule comprising (I); (b) providing an appropriate acceptor molecule for (I); and (c) bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.Alternatively, method (9) may comprise: (a) providing an acceptor molecule comprising (I); (b) providing an appropriate donor molecule for (I); and (c) bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.Preferably, the donor molecule is (I). In method (11), the computational method of designing a fluorescent protein comprises: (a) determining from a three dimensional model of a crystallized fluorescent protein comprising a fluorescent protein with a bound ligand, at least one interacting amino acid of the fluorescent protein that interacts with at least one first chemical moiety of the ligand; and (b) selecting at least one chemical modification of the first chemical moiety to produce a second chemical moiety with a structure to either decrease or increase an interaction between the interacting amino acid and the second chemical moiety compared to the interaction between the interacting amino acid and the first chemical moiety. The computational method further comprises generating the three dimensional model of the crystallized protein comprising (I). Method (11b) selects the first chemical moiety that interacts with at least one of the amino acids fully described in the specification, where the coordinates for the crystal structure if Aequorea-related GFP S65T. The chemical modification enhances hydrogen bonding interaction, charge interaction, hydrophobic interaction, Van Der Waals interaction or dipole interaction between the second chemical moiety and the interacting amino acid, compared to the first chemical moiety and the interacting amino acid. In method (12), determining comprises determining the three dimensional structure of a fluorescent protein with an amino acid sequence at least 80, preferably 95 %, identical to (I-a). The computational method also comprises determining the three dimensional relationship of at least 1500 atoms, which is fully listed in the specification, of the coordinates for the crystal structure if Aequorea-related GFP. In method (14), the fluorescence in the presence of a test chemical is greater than in the absence of the test chemical. Preferably, the polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a genomic polynucleotide. The functional, engineered fluorescent protein is fused to second functional protein. The polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a response element, where the polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a response element in a mammalian cell. Method (15) further comprises comparing the fluorescence of (I) in the sample to the fluorescence of a control engineered green fluorescent protein introduced into a control sample comprising the anion of interest (particularly a halide) at a known concentration. In particular, the sample comprises at least one living cell. In method (16), screening the effects of test compounds

on ion channel activity, comprises: (a) providing a cell comprising (I) and an ion channel of interest; (b) contacting the cell with a test compound; and (c) determining fluorescence from the engineered green fluorescent protein. The method further comprises contacting the cell with a known activator of the ion channel of interest. Furthermore, the method involves comparing the fluorescence of (I) in the cell to the fluorescence of a control engineered GFP introduced into a control cell. Preferably, the ion channel of interest transports halides. Preferred Device: The storage device is a computer readable device that stores code that receives as input the atomic coordinates. The computer readable device is a floppy disk or a hard drive.

USE - (I) is useful as markers for gene expression, tracers of cell lineage or as fusion tags to monitor protein localization within living cells. (I) is particularly useful for coupling engineered fluorescent proteins to antibodies, nucleic acids or other receptors for use in detection assays, e.g. immunoassays or hybridization assays. (I) is also useful for tracking the movement of proteins in cells, or in systems for detecting induction of transcription. (I) is particularly useful for the simultaneous measurement of two or more processes within cells and is also useful as fluorescent energy donors or acceptors, as well as biosensors for detecting anions. (I) is also useful in fluorescence resonance energy transfer (FRET). The crystal structure of the green fluorescent protein is useful for designing mutants having altered fluorescent characteristics. This is particularly useful in identifying amino acids whose substitution alters fluorescent properties of the protein. The crystal structure of the green fluorescent protein is also useful for designing mutants having altered anion binding characteristics. This is particularly useful for identifying amino acids whose substitution alters the specificity and affinity of the binding site to various anions, and for monitoring anion binding and therefore the concentration of the anion.

ADVANTAGE - The present engineered fluorescent protein has varied fluorescent properties and has the ability to respond to ion concentrations via a change in fluorescent characteristics. The functional engineered fluorescent proteins with varied fluorescent characteristics can be easily distinguished from currently existing green and blue fluorescent proteins. The engineered fluorescent proteins enable the simultaneous measurement of two or more processes within cells and can be used as fluorescent energy donors or acceptors, as well as biosensors for detecting anions. The present engineered fluorescent proteins are particularly useful because photodynamic toxicity and auto-fluorescence of cells are significantly reduced at longer wavelengths. In particular, the introduction of the substitution T203X, where X is an aromatic amino acid, results in an increase in the excitation and emission wavelength maxima of Aequorea-related fluorescent proteins. Another primary advantage of fluorescent protein fusion proteins is that they are prepared by normal protein biosynthesis, thus completely avoiding organic synthesis and the requirement for customized unnatural amino acid analogs.

EXAMPLE - No relevant examples given. (181 pages)

L7 ANSWER 11 OF 12 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER:	2000:271897 HCAPLUS
DOCUMENT NUMBER:	132:289597
TITLE:	Engineered fluorescent proteins with altered fluorescence characteristics
INVENTOR(S):	Tsien, Roger Y.; Remington, S. James; Cubitt, Andrew B.; Heim, Roger; Ormo, Mats F.
PATENT ASSIGNEE(S):	The Regents of the University of California, USA
SOURCE:	U.S., 76 pp., Cont.-in-part of U.S. Ser. No. 706,408. CODEN: USXXAM
DOCUMENT TYPE:	Patent
LANGUAGE:	English

FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6054321	A	20000425	US 1997-911825	19970815
US 6124128	A	20000926	US 1996-706408	19960830
CA 2232242	A1	19980219	CA 1997-2232242	19970815
EP 1508574	A2	20050223	EP 2004-24850	19970815
EP 1508574	A3	20051130		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
ES 2231890	T3	20050516	ES 1997-941350	19970815
US 6077707	A	20000620	US 1997-974737	19971119
US 6403374	B1	20020611	US 1999-465142	19991216
US 2003013149	A1	20030116	US 2000-575847	20000519
US 6593135	B2	20030715		
AU 767375	B2	20031106	AU 2001-23196	20010223
US 2003036178	A1	20030220	US 2002-71976	20020205
US 6780975	B2	20040824		
US 2004014128	A1	20040122	US 2003-620099	20030714
AU 2004200425	A1	20040304	AU 2004-200425	20040205
US 2005079525	A1	20050414	US 2004-924232	20040823

PRIORITY APPLN. INFO.:

US 1996-24050P	P	19960816
US 1996-706408	A2	19960830
AU 1997-43277	A3	19970815
EP 1997-941350	A3	19970815
US 1997-911825	A1	19970815
US 1997-974737	A1	19971119
US 1999-465142	A1	19991216
US 2000-575847	A3	20000519
AU 2001-23196	A3	20010223
US 2002-71976	A1	20020205

AB This invention provides methods for engineering functional fluorescent proteins with varied fluorescence characteristics to that of currently existing green and blue fluorescent proteins. In one embodiment, this invention provides a computational method of modeling the three dimensional structure of any other fluorescent protein based on the three dimensional structure of an Aequorea green fluorescent protein (GFP). Amino acid substitutions in polarizable or charged side chains near the p-hydroxycinnamyl group results in modulation of the electronic environment of the chromophore, absorption and fluorescent emission spectrum, and pH sensitivity of the mutant. The three dimensional structure of the S65T GFP mutant was determined at 1.9A resolution Several mutants of Aequorea GFP, T203I, E222G, T203H, T203Y, T203W, and T203Y/S65G/V68L/S72A displayed altered fluorescence characteristics, including suppression of one of the absorbance peaks, and red shifting of excitation and emission peaks.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 12 OF 12 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 95317418 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7796912
TITLE: Green-fluorescent protein mutants with altered fluorescence excitation spectra.
AUTHOR: Ehrig T; O'Kane D J; Prendergast F G
CORPORATE SOURCE: Department of Pharmacology, Mayo Foundation, Rochester, Minnesota 55905, USA.
CONTRACT NUMBER: GM-34847 (NIGMS)
GM-46300 (NIGMS)
SOURCE: FEBS letters, (1995 Jun 26) Vol. 367, No. 2, pp. 163-6.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 17 Aug 1995
Last Updated on STN: 6 Feb 1998
Entered Medline: 3 Aug 1995

AB Using random mutagenesis and visual selection of fluorescent clones, we have isolated a T203I and a E222G mutant of the Aequorea green-fluorescent protein. Each mutant has one of the two fluorescence excitation bands of the wild type deleted and retains the other without a wavelength shift. This finding is consistent with each excitation band corresponding to a distinct spectroscopic state of the chromophore. Both mutations are single amino acid exchanges which in the linear sequence are located remotely from the chromophore but in the folded protein may be situated in its vicinity. We conclude that the mutations influence the fluorescence properties by changing the interactions between the chromophore and its protein environment.

=> d his

(FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007

L1 115659 S GREEN (W)FLUORESCENT
L2 22 S AEQUOREA (W) COERULESCENS
L3 13 S L1 AND L2
L4 7 DUP REM L3 (6 DUPLICATES REMOVED)
L5 5 S "ACGFPL"
L6 20 S "E222G" AND L1
L7 12 DUP REM L6 (8 DUPLICATES REMOVED)

=> s l2 and l6
L8 4 L2 AND L6

=> d 1-4 ibib ab

L8 ANSWER 1 OF 4 MEDLINE on STN
ACCESSION NUMBER: 2003313870 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12693991
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.
AUTHOR: Gurskaya Nadya G; Fradkov Arkady F; Pounkova Natalia I; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Labas Yulii A; Lukyanov Sergey; Lukyanov Konstantin A
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow 117997, Russia.
SOURCE: The Biochemical journal, (2003 Jul 15) Vol. 373, No. Pt 2, pp. 403-8.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY151052; GENBANK-AY233272
ENTRY MONTH: 200308

ENTRY DATE: Entered STN: 8 Jul 2003
Last Updated on STN: 16 Aug 2003
Entered Medline: 15 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222)-->Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Re-introduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L8 ANSWER 2 OF 4 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2003300328 EMBASE
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.
AUTHOR: Gurskaya N.G.; Fradkov A.F.; Pounkova N.I.; Staroverov D.B.; Bulina M.E.; Yanushevich Y.G.; Labas Y.A.; Lukyanov S.; Lukyanov K.A.
CORPORATE SOURCE: K.A. Lukyanov, Shemyakin/Ovchinnikov Inst. B., Miklukho-Maklaya 16/10, Moscow 117997, Russian Federation. kluk@ibch.ru
SOURCE: Biochemical Journal, (15 Jul 2003) Vol. 373, No. 2, pp. 403-408. .
Refs: 25
ISSN: 0264-6021 CODEN: BIJOAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 14 Aug 2003
Last Updated on STN: 14 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222) → Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Reintroduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and β-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L8 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:378716 BIOSIS
DOCUMENT NUMBER: PREV200300378716
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.
AUTHOR(S): Gurskaya, Nadya G.; Fradkov, Arkady F.; Pounkova, Natalia I.; Staroverov, Dmitry B.; Bulina, Maria E.; Yanushevich, Yurii G.; Labas, Yulii A.; Lukyanov, Sergey; Lukyanov, Konstantin A. [Reprint Author]
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow, 117997, Russia
kluk@ibch.ru
SOURCE: Biochemical Journal, (15 July 2003) Vol. 373, No. 2, pp. 403-408. print.
ISSN: 0264-6021.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 20 Aug 2003
Last Updated on STN: 20 Aug 2003
AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu222 fwdarw Gly (E222G) substitution, which removed the evolutionarily invariant Glu222. Reintroduction of Glu222 into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L8 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:655855 HCAPLUS
DOCUMENT NUMBER: 139:376885
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants
AUTHOR(S): Gurskaya, Nadya G.; Fradkov, Arkady F.; Pounkova, Natalia I.; Staroverov, Dmitry B.; Bulina, Maria E.; Yanushevich, Yurii G.; Labas, Yulii A.; Lukyanov, Sergey; Lukyanov, Konstantin A.
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, 117997, Russia
SOURCE: Biochemical Journal (2003), 373(2), 403-408
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have cloned an unusual colorless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu222 → Gly (E222G) substitution, which

removed the evolutionarily invariant Glu222. Reintroduction of Glu222 into the most fluorescent random mutant, named aceGFP, converted it into a colorless protein. This colorless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biol. tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of "humanized" aceGFP and β-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007
L1 115659 S GREEN (W)FLUORESCENT
L2 22 S AEQUOREA (W) COERULESCENS
L3 13 S L1 AND L2
L4 7 DUP REM L3 (6 DUPLICATES REMOVED)
L5 5 S "ACGFPL"
L6 20 S "E222G" AND L1
L7 12 DUP REM L6 (8 DUPLICATES REMOVED)
L8 4 S L2 AND L6

=> s V11I or K101E or 1206A or F64L
L9 302 V11I OR K101E OR 1206A OR F64L

=> s aequorea and l1
L10 3003 AEQUOREA AND L1

=> s l9 and l10
L11 35 L9 AND L10

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12 17 DUP REM L11 (18 DUPLICATES REMOVED)

=> d 1-17 ibib ab

L12 ANSWER 1 OF 17 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-07418 BIOTECHDS
TITLE: New chimeric phosphorylation indicator comprises a first fluorescent protein, phosphoaminoacid binding domain with FHA2 sequence and protein kinase C-phosphorylatable domain, useful for detecting protein kinase C or phosphates; production of a chimeric protein having fluorescence useful for detection of protein-protein interaction and for a general proteomics application
AUTHOR: VIOLIN J D; NEWTON A C; TSIEN R Y; ZHANG J
PATENT ASSIGNEE: VIOLIN J D; NEWTON A C; TSIEN R Y; ZHANG J
PATENT INFO: US 2005026234 3 Feb 2005
APPLICATION INFO: US 2004-857622 28 May 2004
PRIORITY INFO: US 2004-857622 28 May 2004; US 1996-594575 31 Jan 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-141377 [15]
AB DERWENT ABSTRACT:
NOVELTY - A chimeric phosphorylation indicator (I) comprising in operative linkage, a first fluorescent protein, a phosphoaminoacid binding domain with FHA2 sequence comprising a fully defined sequence

(S1) of 81 amino acids as given in the specification, a protein kinase C (PKC)-phosphorylatable domain, and a second fluorescent protein, is new.

DETAILED DESCRIPTION - A chimeric phosphorylation indicator (I) comprising in operative linkage, a first fluorescent protein, a phosphoaminoacid binding domain having FHA2 sequence comprising a fully defined sequence (S1) of 81 amino acids as given in the specification, a protein kinase C (PKC)-phosphorylatable domain, and a second fluorescent protein, where the first and second fluorescent proteins are different and one or more of first and the second fluorescent proteins comprises a non-oligomerizing fluorescent protein. The first and second fluorescent proteins are chosen from green fluorescent proteins (GFPs), red fluorescent proteins (RFPs), and fluorescent proteins related to a GFP or an RFP, a fluorescent protein related to a GFP or related to an RFP that comprises an amino acid sequence having 90% or more sequence homology to a GFP or an RFP. The first and second fluorescent proteins exhibit a detectable resonance energy transfer, when the first fluorescent protein is excited. The PKC-phosphorylatable domain and phosphoaminoacid binding domain do not substantially emit light to excite the second fluorescent protein. INDEPENDENT CLAIMS are also included for the following: (1) a polynucleotide (II) encoding (I); (2) a vector (III) comprising (II); (3) a host cell (IV) comprising (II); and (4) a kit (V) comprising (I) or (II).

BIOTECHNOLOGY - Preferred Indicator: In (I), the PKC-phosphorylatable domain comprises sequence (S2)-(S12) of Arg-Phe-Arg-Arg-Phe-Gln-Thr-Leu-Lys-Ile-Lys-Ala-Lys-Ala, Lys-Lys-Lys-Lys-Lys-Lys-Arg-Phe-Ser-Phe-Lys-Lys-Ser-Phe-Lys-Leu-Ser-Gly-Phe-Ser-Phe-Lys-Lys-Asn-Leu-Leu, Lys-Lys-Arg-Phe-Ser-Phe-Lys-Lys-Phe-Lys-Leu, Lys-Arg-Phe-Ser-Ser-Lys-Lys-Ser-Phe-Lys-Leu-Leu-Ser-Gly-Phe-Ser-Phe-Lys-Lys-Asn-Lys-Lys-Glu-Ala, Lys-Arg-Phe-Ser-Ser-Lys-Lys-Ser-Phe-Lys-Leu-Ser-Gly-Phe-Ser-Phe-Lys-Lys-Ser-Lys-Lys-Glu-Ala, Lys-Lys-Phe-Ser-Ser-Lys-Lys-Pro-Phe-Lys-Leu-Ser-Gly-Phe-Ser-Phe, Glu-Thr-Thr-Ser-Ser-Phe-Lys-Lys-Phe-Phe-Thr-His-Gly-Thr-Ser-Phe-Lys-Lys-Ser-Lys-Glu-Asp-Asp, Lys-Leu-Phe-Ser-Ser-Ser-Gly-Leu-Lys-Lys-Leu-Ser-Gly-Lys-Lys-Gln-Lys-Gly-Lys-Arg-Gly-Gly-Gly, Glu-Gly-Ile-Thr-Pro-Trp-Ala-Ser-Phe-Lys-Lys-Met-Val-Thr-Pro-Lys-Lys-Arg-Val-Arg-Arg-Pro-Ser or Glu-Gly-Val-Ser-Thr-Trp-Glu-Ser-Phe-Lys-Arg-Leu-Val-Thr-Pro-Arg-Lys-Lys-Ser-Lys-Ser-Lys-Leu, preferably (S2). The PKC substrate phosphorylatable domain is adjacent to a polypeptide linker that comprises sequence (S13)-(S16) of Gly-Gly-Ser-Gly-Gly, Gly-His-Gly-Thr-Gly-Ser-Thr-Gly-Ser-Gly-Ser-Ser, Arg-Met-Gly-Ser-Thr-Ser-Gly-Ser-Thr-Lys-Gly-Gln-Leu or Arg-Met-Gly-Ser-Thr-Ser-Gly-Ser-Gly-Lys-Pro-Gly-Ser-Gly-Glu-Gly-Ser-Thr-Lys-Gly-Gln-Leu. The red fluorescent protein is a Discosoma RFP or a fluorescent protein related to Discosoma RFP. The fluorescent protein is a GFP chosen from Aequorea GFP, Renilla GFP, Phialidium GFP, or a fluorescent protein related to an Aequorea GFP, Renilla GFP, and Phialidium GFP. The fluorescent protein related to the Aequorea GFP is a cyan fluorescent protein (CFP), monomeric GFP (mGFP), monomeric CFP (mCFP), yellow fluorescent protein (YFP), monomeric YFP (mYFP), or a spectral variant of the CFP or YFP. The fluorescent protein related to Aequorea GFP is an enhanced GFP (EGFP) (a fully defined sequence (S17) of 239 amino acids as given in the specification), an enhanced CFP (ECFP) (a fully defined sequence (S18) of 239 amino acids as given in the specification), ECFP having amino acids from position 1-227 of (S18), EYFP-Val68Leu/Gln69Lys (a fully defined sequence (S19) of 239 amino acids as given in the specification), enhanced YFP (EYFP) (a fully defined sequence (S20) of 239 amino acids as given in the specification), citrine (S19 with Gln69Met), monomeric GFP (mGFP), monomeric CFP (mCFP) or monomeric YFP (mYFP), where mGFP, mCFP and mYFP comprise a mutation of an amino acid residue corresponding to Ala206, Leu221 or Phe223 of a reference fluorescence protein related to Aequorea GFP. The fluorescent protein comprises a mutation of an amino acid residue corresponding to Ala206Lys, Leu221Lys, Phe223Arg or Leu221Lys or their combinations of a fully defined sequence (S21) of 238 amino acids as given in the specification, (S18) or (S19). (I) comprises,

in an orientation from the amino terminus to carboxy terminus, mCFP, polypeptide linker, FHA2 phosphoaminoacid binding domain (S1), PKC substrate phosphorylatable domain (S2) and having an amino end and carboxy end, where the PKC substrate phosphorylatable domain is flanked on both their amino and carboxy end by a polypeptide linker, and mYFP. The PKC substrate phosphorylatable domain and their flanking polypeptide linkers comprise a sequence (S22) of Gly-Gly-Ser-Gly-Gly-Arg-Phe-Arg-Arg-Phe-Gln-Thr-Leu-Lys-Ile-Lys-Ala-Lys-Ala-Gly-Gly-Ser-Gly-Gly. One or more amino acid of the phosphorylatable domain is phosphorylated. (I) further comprises a cell compartmentalization domain, which is a membrane targeting domain. The membrane targeting domain comprises an amino acid sequence to which a lipid molecule may covalently bound. The membrane targeting domain comprises sequence (S23)-(S25) of Cys-Leu-Leu-Leu, Met-Leu-Cys-Cys-Met-Arg-Arg-Thr-Lys-Gln, or Met-Gly-Cys-Ile-Lys-Ser-Lys-Arg-Lys-Asp-Asn-Leu-Asn-Asp-Asp-Glu. Optionally, the phosphoaminoacid binding domain comprises a fully defined sequence (S26) (FHA1) of 68 amino acids as given in the specification. The polypeptide linker comprises a polypeptide having 3-50, 4-30 or 5-15 amino acid residues. Preferred Polynucleotide: (II) is operatively linked to an expression control sequence e.g. transcription regulatory element, translation regulatory element, or their combinations. Preferred Vector: (III) is an expression vector. Preferred Kit: (V) comprises several of (I). (I) comprises different phosphorylatable domain. (I) comprises different donor molecule, acceptor molecule or both.

USE - (I) is useful for detecting a protein kinase C (PKC) or phosphates in a sample (such as biological sample e.g. cell, tissue sample, or their extracts) which involves contacting the sample with (I), exciting the first fluorescent protein and determining a fluorescence property in the sample, where the presence of kinase or phosphatase in the sample results in a change in the degree of fluorescence resonance energy transfer (FRET). The change in the degree of FRET is an increased or decreased amount of FRET. A change in the degree of FRET is indicative of a PKC or phosphatase in the sample. The phosphorylatable domain is phosphorylated prior to contacting the sample with (I). The above method is performed on an intact cell or tissue sample. (I) further comprises a targeting sequence that has cell compartmentalization domain. The cell compartmentalization domain targets (I) to cell membrane, internal membrane, cytosol, endoplasmic reticulum, mitochondrial matrix, chloroplast lumen, medial trans-golgi cisternae, a lumen of a lysosome, or a lumen of an endosome. The cell compartmentalization domain is a membrane targeting domain. The absence of kinase or phosphatase activity in the sample is due to the presence of kinase inhibitor or phosphatase inhibitor. The kinase inhibitor is a PKC kinase inhibitor. (I) is useful for detecting a kinase inhibitor or phosphatase inhibitor which involves determining a first fluorescent property of (I) in the presence of a kinase or phosphatase, contacting (I) with a composition suspected of being a kinase or phosphatase inhibitor, and determining a second fluorescence property of (I) in the presence of the composition, where a difference in the first and second fluorescent property identifies the composition as a kinase or phosphatase inhibitor. The above method is adapted to high throughput analysis (claimed).

EXAMPLE - The PKA chimeric reporter protein was constructed by fusing the enhanced cyan fluorescence protein (1-227; ECFP; SEQ ID NO:6; K26R/F64L/S65T/Y66W/N146I/M153T/V163A/N164H), a truncated version of 14-3-3tau, a modified kemptide and citrine, which is an improved yellow fluorescence protein having a sequence as set forth in SEQ ID NO:10, except containing a Q69M mutation. 14-3-3tau (1-232) was amplified using the cDNA of 14-3-3tau (see GenBank Accession Number D87662) in pcDNA3 vector as the template. For PCR, the forward primer had the sequence (SEQ ID NO:26) 5'-GGGCATGCATATGGAGAAGACTGAGCTGATCCAG-3', and incorporates a Sph I site. The reverse primer had the sequence (SEQ ID NO:27) 5'-CGCGGAGCTCGCTGCCGCCG-GTGCCGCCCAGGCTGGCGCGACGGAGGCTGCCGCCGGTGCCG CCTGCAG- AGT CTGATGTCCAAAGTGTTAGG-3-', which introduces a short linker peptide (AGGTGGS; SEQ ID NO:19), the kemptide sequence (LRRASLG; SEQ ID

NO:32), a second short linker peptide (GTGGSEL; SEQ ID NO:21) and a Sac I site. PCR was performed using 50 ng of template, 300 nM of each primer, 500 nM of each dNTP, 2.5 Unit of Taq polymerase (Gibco) in 1xPCR reaction buffer (Boehringer Mannheim) with nanopure water (50microl total volume). PCR was performed as follows: 95degreesC., 5 min; 2 cycles of (95degreesC., 1 min; 40degreesC., 1 min; 72degreesC., 2.5 min); 5 cycles of (95degreesC., 1 min; 43degreesC., 1 min; 72degreesC., 2.5 min); 5 cycles of (95degreesC., 1 min; 45degreesC., 1 min; 72degreesC., 2.5 min); 15 cycles of (95degreesC., 1 min; 52degreesC., 1 min; 72degreesC., 2.5 min; 72degreesC., 7 min), then hold at 25degreesC. The amplification product was purified using the Qiagen gel purification kit, then digested with Sph I and Sac I overnight. The digested mixture was purified using Qiagen PCR purification kit, and the purified fragment was ligated into Sph I/Sac I-digested pRSET.sub.B (Invitrogen) containing the cDNA sequence for ECFP and citrine (from Yellow cameleon 3.3). The construct was within the Bam HI/Eco RI sites of pRSET.sub.B, and is behind a polyhistidine tag for bacterial expression. The resulting plasmid was amplified, sequenced and mutagenized using the QuickChange.TM. site-directed mutagenesis kit (Stratagene) to introduce one amino acid change in the kemptide sequence, generating the plasmid C4kY2.1-pRSETB. For mammalian expression, both C4kY2.1 and C4kY2.1(S475A) were cloned into the vector pcDNA3 behind a Kozak sequence for mammalian expression. (68 pages)

L12 ANSWER 2 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:1075799 HCAPLUS
DOCUMENT NUMBER: 142:129412
TITLE: Room temperature spectrally resolved single-molecule spectroscopy reveals new spectral forms and photophysical versatility of Aequorea green fluorescent protein variants
AUTHOR(S): Blum, Christian; Meixner, Alfred J.; Subramaniam, Vinod
CORPORATE SOURCE: Physical Chemistry I, University of Siegen, Siegen, Germany
SOURCE: Biophysical Journal (2004), 87(6), 4172-4179
CODEN: BIOJAU; ISSN: 0006-3495
PUBLISHER: Biophysical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB It is known from ensemble spectroscopy at cryogenic temps. that variants of the Aequorea green fluorescent protein (GFP) occur in interconvertible spectroscopically distinct forms which are obscured in ensemble room temperature spectroscopy. By analyzing the fluorescence of the GFP variants EYFP and EGFP by spectrally resolved single-mol. spectroscopy we were able to observe spectroscopically different forms of the proteins and to dynamically monitor transitions between these forms at room temperature In addition to the predominant EYFP B-form we have observed the blue-shifted I-form thus far only seen at cryogenic temps. and have followed transitions between these forms. Further we have identified for EYFP and for EGFP three more, so far unknown, forms with red-shifted fluorescence. Transitions between the predominant forms and the red-shifted forms show a dark time which indicates the existence of a nonfluorescent intermediate. The spectral position of the newly-identified red-shifted forms and their formation via a nonfluorescent intermediate hint that these states may account for the possible photoactivation observed in bulk expts. The comparison of the single-protein spectra of the red-shifted EYFP and EGFP forms with single-mol. fluorescence spectra of DsRed suggest that these new forms possibly originate from an extended chromophoric π-system analogous to the DsRed chromophore.
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 3 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:832828 HCAPLUS
DOCUMENT NUMBER: 137:334476
TITLE: Preparation of green fluorescent protein mutants with enhanced fluorescence for use as reporter proteins
INVENTOR(S): Stubbs, Simon Lawrence John; Jones, Anne Elizabeth; Michael, Nigel Paul; Thomas, Nicholas
PATENT ASSIGNEE(S): Amersham Biosciences UK Ltd., UK
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002085936	A1	20021031	WO 2001-GB4363	20010928
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
GB 2374868	A	20021030	GB 2001-23288	20010928
GB 2374868	B	20030709		
CA 2445035	A1	20021031	CA 2001-2445035	20010928
AU 2001292040	A1	20021105	AU 2001-292040	20010928
US 2003175859	A1	20030918	US 2001-967301	20010928
US 6919186	B2	20050719		
EP 1381625	A1	20040121	EP 2001-972260	20010928
EP 1381625	B1	20041124		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
AT 283283	T	20041215	AT 2001-972260	20010928
JP 2005502323	T	20050127	JP 2002-583462	20010928
ES 2233690	T3	20050616	ES 2001-1972260	20010928
US 2004138420	A1	20040715	US 2004-757624	20040114
US 7091317	B2	20060815		
US 2006036078	A1	20060216	US 2005-251209	20051014

PRIORITY APPLN. INFO.:		
GB 2001-9858	A	20010423
US 2001-967301	A3	20010928
WO 2001-GB4363	W	20010928
US 2004-757624	A3	20040114

AB The present invention provides novel engineered derivs. of green fluorescent protein (GFP) which have an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein. The modified GFPs exhibit enhanced fluorescence relative to wtGFP when expressed in non-homologous cells at temps. above 30 °C, and when excited at about 490 nm compared to the parent proteins, i.e. wtGFP. An example of a preferred protein is F64L-S175G-E222G-GFP. The modified GFPs provide a means for detecting GFP reporters in mammalian cells at lower levels of expression and/or increased sensitivity relative to wtGFP. This greatly improves the usefulness of fluorescent proteins in studying cellular functions in living cells.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 4 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:841090 HCAPLUS
DOCUMENT NUMBER: 137:334472
TITLE: Modified green fluorescent protein E2GFP and cDNA and use of E2GFP in optical memory circuits
INVENTOR(S): Beltram, Fabio; Cinelli, Riccardo; Ferrari, Aldo; Giacca, Mauro; Pellegrini, Vittorio; Tyagi, Mudit
PATENT ASSIGNEE(S): Istituto Nazionale per la Fisica della Materia, Italy; International Centre for Genetic Engineering and Biotechnology
SOURCE: Ital. Appl., 53 pp.
CODEN: ITXXCZ
DOCUMENT TYPE: Patent
LANGUAGE: Italian
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
IT 2000TO0772	A1	20020204	IT 2000-TO772	20000802
IT 1320791	B1	20031210		
PRIORITY APPLN. INFO.:			IT 2000-TO772	20000802

AB E2GFP, which differs from wild-type GFP by the 3 substitutions F64L, S65T, and T203Y, and cDNA encoding E2GFP, are disclosed. E2GFP may be used in optical memory circuits. Thus, the cDNA for an E2GFP-glutathione S transferase fusion protein was expressed in Escherichia coli. The fluorescent properties of single mols. this fusion protein in solution or in a polyacrylamide film was observed Loss of fluorescence after laser illumination at 476 nm was reversed by laser illumination at 305 nm.

L12 ANSWER 5 OF 17 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002004119 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11753368
TITLE: A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications.
AUTHOR: Nagai Takeharu; Ibata Keiji; Park Eun Sun; Kubota Mie; Mikoshiba Katsuhiko; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama, 351-0198, Japan.
SOURCE: Nature biotechnology, (2002 Jan) Vol. 20, No. 1, pp. 87-90. Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 2 Jan 2002
Last Updated on STN: 17 Mar 2002
Entered Medline: 15 Mar 2002

AB The green fluorescent protein (GFP) from the jellyfish Aequorea victoria has provided a myriad of applications for biological systems. Over the last several years, mutagenesis studies have improved folding properties of GFP (refs 1,2). However, slow maturation is still a big obstacle to the use of GFP variants for visualization. These problems are exacerbated when GFP variants are expressed at 37 degrees C and/or targeted to certain organelles. Thus, obtaining GFP variants that mature more efficiently is crucial for the development of expanded research applications. Among Aequorea GFP variants, yellow fluorescent proteins (YFPs) are relatively acid-sensitive, and uniquely quenched by chloride ion (Cl-). For YFP to be fully and stably

fluorescent, mutations that decrease the sensitivity to both pH and Cl- are desired. Here we describe the development of an improved version of YFP named "Venus". Venus contains a novel mutation, F46L, which at 37 degrees C greatly accelerates oxidation of the chromophore, the rate-limiting step of maturation. As a result of other mutations, F64L/M153T/V163A/S175G, Venus folds well and is relatively tolerant of exposure to acidosis and Cl-. We succeeded in efficiently targeting a neuropeptide Y-Venus fusion protein to the dense-core granules of PC12 cells. Its secretion was readily monitored by measuring release of fluorescence into the medium. The use of Venus as an acceptor allowed early detection of reliable signals of fluorescence resonance energy transfer (FRET) for Ca2+ measurements in brain slices. With the improved speed and efficiency of maturation and the increased resistance to environment, Venus will enable fluorescent labelings that were not possible before.

L12 ANSWER 6 OF 17 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-06807 BIOTECHDS
TITLE: New long wavelength engineered fluorescent proteins, useful as markers for gene expression, tracers of cell lineage or as fusion tags to monitor protein localization, or in detection assays, e.g. immunoassays or hybridization assays; genetically engineered protein, vector expression in host cell and DNA probe useful for designing mutant strain with altered anion binding and gene expression marker
AUTHOR: WACHTER R; REMINGTON S J
PATENT ASSIGNEE: UNIV OREGON STATE
PATENT INFO: WO 2001090147 29 Nov 2001
APPLICATION INFO: WO 2000-US16149 19 May 2000
PRIORITY INFO: US 2000-575847 19 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-083084 [11]
AB DERWENT ABSTRACT:
NOVELTY - A functional engineered fluorescent protein (I), whose is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (A-GFP) having a 238 residue amino acid sequence (I-a), fully defined in the specification, is new.
DETAILED DESCRIPTION - A functional engineered fluorescent protein (I), whose is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (A-GFP) having a 238 residue amino acid sequence (I-a), fully defined in the specification, is new. (I) has an amino acid sequence that: (a) differs from (I-a) by at least the amino acid substitution T203X; or (b) differs from (I-a) by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, 1167, Q183, N185, L220, E222 (not E222G) or V224. (I) has a different fluorescent property than A-GFP. X = an aromatic amino acid selected from H, Y, W or F. INDEPENDENT CLAIMS are also included for the following: (1) nucleic acid molecules comprising a nucleotide sequence encoding (I); (2) expression vectors comprising expression control sequences operatively linked to the nucleic acid molecule comprising a sequence encoding (I); (3) host cells comprising: (a) recombinant host cells comprising the expression vectors; or (b) (I) whose amino acid sequence differs from (I-a) by at least one first substitution at position T203, and at least one second substitution at position H148; (4) fluorescently labeled antibodies comprising antibodies coupled to (I); (5) nucleic acid molecules comprising nucleotide sequences encoding the antibodies fused to nucleotide sequences encoding (I); (6) fluorescently labeled nucleic acid probes comprising a nucleic acid probes coupled to (I); (7) determining if a mixture contains a target comprising: (a) contacting the mixture with the fluorescently labeled probes; and(b) determining if the target has bound to the probe; (8) engineering (I), which has a fluorescent property different from A-GFP; (9) producing fluorescence resonance energy transfers; (10)

protein comprising (I), where the crystal diffracts with at least a 2.0-3.0 Angstrom resolution; (11) a computational method of designing a fluorescent protein; (12) a computational method of modeling the three dimensional structure of a fluorescent protein by determining a three dimensional relationship between at least two atoms listed in the atomic coordinates fully described in the specification; (13) a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from those atomic coordinates fully listed in the specification; (14) identifying a test chemical, comprising: (a) contacting a test chemical with a sample containing a biological entity labeled with (I) or a polynucleotide encoding (I); and (b) detecting fluorescence of (I); (15) determining the presence of an anion of interest in a sample, comprising: (a) introducing (I) into a sample; and (b) determining the fluorescence of (I); and (16) screening the effects of test compounds on ion channel activity.

BIOTECHNOLOGY - Preferred Nucleic Acid: The amino acid sequence further comprises a mutation fully described in the specification, e.g. Y145F, V163A, N146I, M153T, V163A, N212K, I123V, Y145H, H148R, M153T, S65G, S72A, K79R or T203Y. The nucleic acid sequence encoding the protein differs from a 717 base pair sequence, fully defined in the specification by the substitution of at least one codon by a preferred mammalian codon. The nucleic acid molecule encodes a fusion protein, which comprises a polypeptide of interest and (I). Preferred Host Cell: The recombinant host cell is a prokaryotic cell or a eukaryotic cell. Preferred Protein: The amino acid sequence further comprises a substitution at S65, where the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. The amino acid sequence differs by no more than the substitutions S65T/T203H, S65T/T203Y, S72A/F64L/S65G/T203Y, S72A/S65G/V68L/T203Y, S65G/V68L/Q69K/S72A/T203Y, S65G/S72A/T203Y or S65G/S72A/T203W. The amino acid sequence further comprises a substitution at Y66, where the substitution is selected from Y66H, Y66F and Y66W. The amino acid sequence further comprises a folding mutation. The fusion protein comprises a polypeptide of interest and (I). Preferably, the fusion protein comprises the antibody fused to (I). In particular, the amino acid substitution comprises: (a) L42X, X is C, F, H, W or Y; (b) V6IX, X is F, Y, H or C; (c) T62X, X is A, V, F, S, D, N, Q, Y, H or C; (d) V68X, X is F, Y or H; (e) Q69X, X is K, R, E or G; (f) Q94X, X is D, E, H, K or N; (g) N121X, X is F, H, W or Y; (h) Y145X, X is W, C, F, L, E, H, K or Q; (i) H148X, X is F, Y, N, K, Q or R; (j) V150X, X is F, Y or H; (k) F165X, X is H, Q, W or Y; (l) I167X, X is F, Y or H; (m) Q183X, X is H, Y, E or K; (n) N185X, X is D, E, H, K or Q; (o) L220X, X is H, N, Q or T; (p) E222X, X is N and Q; or (q) V224X, X is H, N, Q, T, F, W or Y. At least an amino acid substitution is located no more than 0.5 nm from the chromophore of the engineered fluorescent protein, where the substitution alters the electronic environment of the chromophore, and where (I) has a different fluorescent property than A-GFP. The fluorescent protein, which comprises the crystal, has at least 200 amino acids, a completeness value of at least 80 % and has a crystal stability within 0.5 % of its unit cell dimensions. In particular, the crystal has the following unit cell dimensions in angstroms: a = 51.8, b = 62.8 and c = 70.7, with a space group of P2 2 2 and an alpha angle of 90.00 degrees, a beta angle of 90.00 degrees and a gamma angle of 90.00 degrees. The crystal has a diffraction limit, where 90 % or greater of the potential reflections can be used to determine the coordinates of the atoms. The substitution at Q69 is selected from K, R, E and G, where the amino acid sequence further comprises a function mutation at S65. The substitution at E222 is selected from N and Q, where the amino acid sequence further comprises a function mutation at F64. The substitution at Y145 is selected from W, C, F, L, E, H, K and Q, where the amino acid sequence further comprises a function mutation at Y66. The nucleotide sequence encodes a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of A-GFP and which differs from (I-a) by: (a) at least one first substitution at position T203, where the substitution consists of H, Y, W or F; and (b)

at least one second substitution at position H148, where (I) has a different fluorescent property than Aequorea green fluorescent protein. At least one second substitution at position H148 is selected from H148R, H148G, H148Q, H148A, H148N and H148K. At least one second substitution at position H148 is H148Q. At least one second substitution at position H148 is H148G. At least one second substitution at position H148 is H148R. (I) further comprises at least one third substitution at position V150, where the substitution at position V150 consists of A, C, M, G, L, Q, S, T and N. (I) further comprises at least one third substitution at position V163, where the at least one third substitution at position V163 is selected from A, C, M, G, L, Q, S, T and N. (I) further comprises at least one-third substitution at position Q69, which consist of N, S, T and V. (I) may further comprise at least one-third substitution at position 'V152, which comprises A, C, M, G, L, V, F, S, T, Q or N. (I) also comprises at least one third substitution at position F165, which consists of Y, L and W. (I) further comprises at least one third substitution at position H181, which consists of K, R, F, Y and W. (I) further comprises at least one third substitution at position L201, where the substitution is selected from A, C, M, G, S, T ,Q, N, V and I. Preferred Method: In method (7), the target is bound to a solid matrix. In method (8), engineering a functional engineered fluorescent protein having a fluorescent property different than A-GFP comprises substituting an amino acid that is located no more than 0.5 nm from any atom in the chromophore of an Aequorea-related GFP with another amino acid; where the substitution alters a fluorescent property of the protein. The amino acid substitution alters the electronic environment of the chromophore. Engineering (I) may also comprise substituting amino acids in a loop domain of an Aequorea-related GFP with amino acids to create a consensus sequence for phosphorylation or for proteolysis. In method (9), producing fluorescence resonance energy transfer comprises: (a) providing a donor molecule comprising (I); (b) providing an appropriate acceptor molecule for (I); and (c) bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.Alternatively, method (9) may comprise: (a) providing an acceptor molecule comprising (I); (b) providing an appropriate donor molecule for (I); and (c) bringing the donor molecule and the acceptor molecule into sufficiently close contact to allow fluorescence resonance energy transfer.Preferably, the donor molecule is (I). In method (11), the computational method of designing a fluorescent protein comprises: (a) determining from a three dimensional model of a crystallized fluorescent protein comprising a fluorescent protein with a bound ligand, at least one interacting amino acid of the fluorescent protein that interacts with at least one first chemical moiety of the ligand; and (b) selecting at least one chemical modification of the first chemical moiety to produce a second chemical moiety with a structure to either decrease or increase an interaction between the interacting amino acid and the second chemical moiety compared to the interaction between the interacting amino acid and the first chemical moiety. The computational method further comprises generating the three dimensional model of the crystallized protein comprising (I). Method (11b) selects the first chemical moiety that interacts with at least one of the amino acids fully described in the specification, where the coordinates for the crystal structure if Aequorea-related GFP S65T. The chemical modification enhances hydrogen bonding interaction, charge interaction, hydrophobic interaction, Van Der Waals interaction or dipole interaction between the second chemical moiety and the interacting amino acid, compared to the first chemical moiety and the interacting amino acid. In method (12), determining comprises determining the three dimensional structure of a fluorescent protein with an amino acid sequence at least 80, preferably 95 %, identical to (I-a). The computational method also comprises determining the three dimensional relationship of at least 1500 atoms, which is fully listed in the specification, of the coordinates for the crystal structure if Aequorea-related GFP. In method (14),

the fluorescence in the presence of a test chemical is greater than in the absence of the test chemical. Preferably, the polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a genomic polynucleotide. The functional, engineered fluorescent protein is fused to second functional protein. The polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a response element, where the polynucleotide encoding the functional, engineered fluorescent protein is operatively linked to a response element in a mammalian cell. Method (15) further comprises comparing the fluorescence of (I) in the sample to the fluorescence of a control engineered green fluorescent protein introduced into a control sample comprising the anion of interest (particularly a halide) at a known concentration. In particular, the sample comprises at least one living cell. In method (16), screening the effects of test compounds on ion channel activity, comprises: (a) providing a cell comprising (I) and an ion channel of interest; (b) contacting the cell with a test compound; and (c) determining fluorescence from the engineered green fluorescent protein. The method further comprises contacting the cell with a known activator of the ion channel of interest. Furthermore, the method involves comparing the fluorescence of (I) in the cell to the fluorescence of a control engineered GFP introduced into a control cell. Preferably, the ion channel of interest transports halides. Preferred Device: The storage device is a computer readable device that stores code that receives as input the atomic coordinates. The computer readable device is a floppy disk or a hard drive.

USE - (I) is useful as markers for gene expression, tracers of cell lineage or as fusion tags to monitor protein localization within living cells. (I) is particularly useful for coupling engineered fluorescent proteins to antibodies, nucleic acids or other receptors for use in detection assays, e.g. immunoassays or hybridization assays. (I) is also useful for tracking the movement of proteins in cells, or in systems for detecting induction of transcription. (I) is particularly useful for the simultaneous measurement of two or more processes within cells and is also useful as fluorescent energy donors or acceptors, as well as biosensors for detecting anions. (I) is also useful in fluorescence resonance energy transfer (FRET). The crystal structure of the green fluorescent protein is useful for designing mutants having altered fluorescent characteristics. This is particularly useful in identifying amino acids whose substitution alters fluorescent properties of the protein. The crystal structure of the green fluorescent protein is also useful for designing mutants having altered anion binding characteristics. This is particularly useful for identifying amino acids whose substitution alters the specificity and affinity of the binding site to various anions, and for monitoring anion binding and therefore the concentration of the anion.

ADVANTAGE - The present engineered fluorescent protein has varied fluorescent properties and has the ability to respond to ion concentrations via a change in fluorescent characteristics. The functional engineered fluorescent proteins with varied fluorescent characteristics can be easily distinguished from currently existing green and blue fluorescent proteins. The engineered fluorescent proteins enable the simultaneous measurement of two or more processes within cells and can be used as fluorescent energy donors or acceptors, as well as biosensors for detecting anions. The present engineered fluorescent proteins are particularly useful because photodynamic toxicity and auto-fluorescence of cells are significantly reduced at longer wavelengths. In particular, the introduction of the substitution T203X, where X is an aromatic amino acid, results in an increase in the excitation and emission wavelength maxima of Aequorea-related fluorescent proteins. Another primary advantage of fluorescent protein fusion proteins is that they are prepared by normal protein biosynthesis, thus completely avoiding organic synthesis and the requirement for customized unnatural amino acid analogs.

EXAMPLE - No relevant examples given.(181 pages)

L12 ANSWER 7 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:935638 HCAPLUS
DOCUMENT NUMBER: 136:66606
TITLE: Novel fluorescent proteins derived from green fluorescent protein
INVENTOR(S): Bjorn, Sara P.; Pagliaro, Len; Thastrup, Ole
PATENT ASSIGNEE(S): Bioimage AS, Den.
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001098338	A2	20011227	WO 2001-EP6848	20010618
WO 2001098338	A3	20020510		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2410413	A1	20011227	CA 2001-2410413	20010618
EP 1299414	A2	20030409	EP 2001-957861	20010618
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2005518180	T	20050623	JP 2002-504293	20010618
US 2002177189	A1	20021128	US 2001-887784	20010619
US 7001986	B2	20060221		
US 2004072995	A1	20040415	US 2003-296953	20030902
US 2006051843	A1	20060309	US 2005-206904	20050819
JP 2007075122	A	20070329	JP 2006-304095	20061109

PRIORITY APPLN. INFO.:	DK 2000-953	A	20000619
	US 2000-212681P	P	20000620
	DK 2001-739	A	20010510
	US 2001-290170P	P	20010510
	JP 2002-504293	A3	20010618
	WO 2001-EP6848	W	20010618
	US 2001-887784	A3	20010619

AB A GFP with an F64L mutation and an E222G mutation is provided. This GFP has a bigger Stokes shift compared to other GFPs making it very suitable for high throughput screening due to a better resolution This GFP also has an excitation maximum between the yellow GFP and the cyan GFP allowing for cleaner band separation when used together with those GFPs.

L12 ANSWER 8 OF 17 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 2
ACCESSION NUMBER: 2001:540696 SCISEARCH
THE GENUINE ARTICLE: 448MH
TITLE: Engineering single-molecule fluorescence dynamics for advanced biomolecular applications
AUTHOR: Cinelli R A G (Reprint); Ferrari A; Pellegrini V; Signorelli A; Tyagi M; Giacca M; Beltram F
CORPORATE SOURCE: INFM, NEST, Piazza Cavalieri 7, I-56126 Pisa, Italy (Reprint); INFM, NEST, I-56126 Pisa, Italy; Scuola Normale Super Pisa, I-56126 Pisa, Italy; Int Ctr Genet Engn & Biotechnol, Mol Med Lab, I-34012 Trieste, Italy

COUNTRY OF AUTHOR: Italy
SOURCE: AUSTRALIAN JOURNAL OF CHEMISTRY, (2001) Vol. 54, No. 2, pp. 107-111.
ISSN: 0004-9425.
PUBLISHER: C S I R O PUBLISHING, 150 OXFORD ST, PO BOX 1139, COLLINGWOOD, VICTORIA 3066, AUSTRALIA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 36
ENTRY DATE: Entered STN: 20 Jul 2001
Last Updated on STN: 20 Jul 2001
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The green fluorescent protein (GFP) of the Aequorea victoria jellyfish is a uniquely fluorescent label that is opening the way to advanced biomolecular studies. Fundamental processes on a nanoscopic scale can, in fact, be directly addressed by imaging and spectroscopy of GFP-tagged molecules. To this end, the design of labels with novel spectral and dynamic properties plays an important role to enhance GFP applicability. We investigated and engineered the fluorescence dynamics of two GFP mutants, the F64L/S65T and the F64L/S65T/T203Y, down to the single-molecule level in order to evaluate their suitability for a broad range of experimental systems. We shall argue that the first mutant is suitable for quantitative fluorescence microscopy and displays fluorescence almost unaffected by the environment, while the second offers the important additional advantage of undergoing photobleaching reversal after ultraviolet illumination. As a consequence, the latter mutant is a viable candidate for applications when prolonged imaging is required. As an example of novel possibilities provided by GFP technology, we shall show studies of HIV-1 Tat activity in cells and, in particular, we shall demonstrate Tat interaction with cyclin T1 by fluorescence-resonance energy transfer.

L12 ANSWER 9 OF 17 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 3
ACCESSION NUMBER: 2002:15705 SCISEARCH
THE GENUINE ARTICLE: 504QV
TITLE: Multiphoton molecular spectroscopy and excited-state dynamics of enhanced green fluorescent protein (EGFP): acid-base specificity
AUTHOR: Heikal A A; Hess S T; Webb W W (Reprint)
CORPORATE SOURCE: Cornell Univ, Sch Appl & Engn Phys, 223 Clark Hall, Ithaca, NY 14853 USA (Reprint); Cornell Univ, Sch Appl & Engn Phys, Ithaca, NY 14853 USA
COUNTRY OF AUTHOR: USA
SOURCE: CHEMICAL PHYSICS, (1 DEC 2001) Vol. 274, No. 1, pp. 37-55.
ISSN: 0301-0104.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 47
ENTRY DATE: Entered STN: 11 Jan 2002
Last Updated on STN: 11 Jan 2002
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Green fluorescent protein (GFP), isolated from Aequorea victoria jellyfish, has been used extensively as a noninvasive intracellular pH indicator and site-specific fluorescent marker in biochemistry, cell biology, and molecular genetics. Numerous mutations, aimed at optimizing spectroscopic and thermodynamic properties of GFP, have been created for different applications. Fluorescence correlation spectroscopy (FCS) reveals that the enhanced green fluorescent protein mutant (EGFP; S65T/F64L) undergoes external proton exchange with the buffer on similar to 45-300 mus time scale with pK(a) = 5.8 +/- 0.1 [Proc. Natl. Acad. Sci. USA 95 (1998)

13573]. This contribution represents a comprehensive characterization of pH and excitation mode (wavelength, one and two photon (2P)) effects on the spectroscopy, excited-state dynamics, and rotational mobility of EGFP aiming at elucidating the significant electronic states of this molecular system. EGFP exhibits a large 2P action cross-section and, therefore, is well suited for intracellular imaging using 2P fluorescence microscopy. (C) 2001 Published by Elsevier Science B.V.

L12 ANSWER 10 OF 17 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:1108 BIOSIS
DOCUMENT NUMBER: PREV200000001108
TITLE: A novel mutant of green fluorescent protein with enhanced sensitivity for microanalysis at 488 nm excitation.
AUTHOR(S): Ito, Yoichiro; Suzuki, Miho; Husimi, Yuzuru [Reprint author]
CORPORATE SOURCE: Department of Functional Materials Science, Saitama University, Urawa, 338-8570, Japan
SOURCE: Biochemical and Biophysical Research Communications, (Oct. 22, 1999) Vol. 264, No. 2, pp. 556-560. print.
CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Dec 1999
Last Updated on STN: 31 Dec 2001

AB Green fluorescent protein (GFP) has been utilized as a powerful reporter of gene expression and protein localization in cells. We discovered a mutant carrying point mutation S208L from a UV-excitable GFP (F99S/M153T/V163A). It had the enhanced fluorescence intensity. Introduction of the red-shifted mutations (F64L/S65T) to this mutant led to the GFP having the brightest mutants reported which were expressed in Escherichia coli and excited at 488 nm. The relative fluorescence intensities to that of wild-type GFP and GFPuv were increased about 120- and 10-fold, respectively. It was shown that the S208L mutation contributes to both a higher intrinsic brightness of GFP and a higher expression level in E. coli.

L12 ANSWER 11 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1998:685114 HCAPLUS
DOCUMENT NUMBER: 129:313128
TITLE: Characterizing a cellular response to a stimulus using a lumiphore genetically modified with a signal pathway component
INVENTOR(S): Thastrup, Ole; Petersen Bjorn, Sara; Tullin, Soren; Kasper, Almholt; Scudder, Kurt
PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.
SOURCE: PCT Int. Appl., 327 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9845704	A2	19981015	WO 1998-DK145	19980407
WO 9845704	A3	19990422		

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES,

FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

CA 2286293	A1	19981015	CA 1998-2286293	19980407
CA 2286293	C	20040406		
CA 2450698	A1	19981015	CA 1998-2450698	19980407
AU 9868209	A	19981030	AU 1998-68209	19980407
EP 986753	A2	20000322	EP 1998-913541	19980407
EP 986753	B1	20020327		
EP 986753	B2	20050810		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, LT, LV, FI				
JP 2001522454	T	20011113	JP 1998-542276	19980407
AT 215227	T	20020415	AT 1998-913541	19980407
EP 1199564	A2	20020424	EP 2001-204477	19980407
EP 1199564	A3	20020508		
EP 1199564	B1	20040623		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
PT 986753	T	20020930	PT 1998-913541	19980407
ES 2173573	T3	20021016	ES 1998-913541	19980407
EP 1435519	A1	20040707	EP 2003-28919	19980407
EP 1435519	B1	20070214		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
AT 269975	T	20040715	AT 2001-204477	19980407
PT 1199564	T	20041029	PT 2001-204477	19980407
ES 2191575	T3	20050201	ES 2001-204477	19980407
AT 354089	T	20070315	AT 2003-28919	19980407
US 6518021	B1	20030211	US 1999-417197	19991007
US 2003082564	A1	20030501	US 2002-72036	20020205

PRIORITY APPLN. INFO.:	DK 1997-392	A 19970407
	CA 1998-2286293	A3 19980407
	EP 1998-913541	A3 19980407
	EP 2001-204477	A3 19980407
	WO 1998-DK145	W 19980407
	US 1999-417197	A3 19991007

AB Cells are genetically modified to express a luminophore, e.g., a modified (F64L, S65T, Y66H) Green Fluorescent Protein (GFP, EGFP) coupled to a component of an intracellular signaling pathway such as a transcription factor, a cGMP- or cAMP-dependent protein kinase, a cyclin-, calmodulin- or phospholipid-dependent or mitogen-activated serine/threonine protein kinase, a tyrosine protein kinase, or a protein phosphatase (e.g. PKA, PKC, Erk, Smad, VASP, actin, p38, Jnk1, PKG, IkappaB, CDK2, Grk5, Zap70, p85, protein-tyrosine phosphatase 1C, Stat5, NFAT, NFkappaB, RhoA, PKB). An influence modulates the intracellular signaling pathway in such a way that the luminophore is being redistributed or translocated with the component in living cells in a manner exptl. determined to be correlated to the degree of the influence. Measurement of redistribution is performed by recording of light intensity, fluorescence lifetime, polarization, wavelength shift, resonance energy transfer, or other properties by an apparatus consisting of e.g. a fluorescence microscope and a CCD camera. Data stored as digital images are processed to nos. representing the degree of redistribution. The method can be used as a screening program for identifying a compound that modulates a component and is capable of treating a disease related to the function of the component.

L12 ANSWER 12 OF 17 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER:	1998:590689 HCAPLUS
DOCUMENT NUMBER:	129:227822
TITLE:	FACS-optimized mutants of the green fluorescent protein (GFP)
INVENTOR(S):	Cormack, Brendan P.; Valdivia, Raphael H.; Falkow, Stanley

PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, USA
SOURCE: U.S., 15 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5804387	A	19980908	US 1997-791332	19970131
US 5994077	A	19991130	US 1997-926556	19970910
US 6090919	A	20000718	US 1998-135418	19980817
PRIORITY APPLN. INFO.:			US 1996-10960P	P 19960201
			US 1997-791332	A2 19970131

AB Three classes of green fluorescent protein (GFP) mutants are described which have single excitation maxima around 488 nm and which are brighter than lid-type GFP following 488 nm excitation. GFPmut1 has a double substitution: F64L, S65T; GFPmut2 has a triple substitution: S65A, V68L, S72A; and GFPmut3 is characterized by the double substitution: S65G, S72A. The excitation maxima of the three mutants are at 488 nm, 481 nm and 501 nm resp. The fluorescence intensities following excitation at 488 nm are an order of magnitude higher than that of wild-type GFP excited at 488 nm in E. coli. The expression of GFP is observable minutes after induction.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 13 OF 17 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1998332512 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9665694
TITLE: pH-dependent fluorescence of a heterologously expressed Aequorea green fluorescent protein mutant: in situ spectral characteristics and applicability to intracellular pH estimation.
AUTHOR: Robey R B; Ruiz O; Santos A V; Ma J; Kear F; Wang L J; Li C J; Bernardo A A; Arruda J A
CORPORATE SOURCE: Department of Medicine, Section of Nephrology, University of Illinois at Chicago College of Medicine 60612, USA.. RBRobey@uic.edu
SOURCE: Biochemistry, (1998 Jul 14) Vol. 37, No. 28, pp. 9894-901. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 20 Aug 1998
Last Updated on STN: 20 Aug 1998
Entered Medline: 12 Aug 1998
AB The green fluorescent protein of Aequorea victoria (GFP) is a natural peptide chromophore without substrate or cofactor requirements for fluorescence. In vitro, a recombinant F64L/S65T GFP mutant (GFPmut1) exhibited pH sensitive fluorescence within the physiologic range. When heterologously expressed in BS-C-1 cells or rabbit proximal tubule cells, uniform cytosolic and nuclear fluorescence was observed. Cytosolic fluorescence constituted over 80% of the total. Excitation scanning of transfected cells revealed two GFPmut1-specific regions that were pH-sensitive over the physiologic range, and each region exhibited a unique pH "bias" in fluorescence emission. Excitation at or near the expected maximum of 488 nm (region II) uniformly resulted in fluorescence that was preferentially altered at

acidic pH. In contrast, a novel "wild-type" excitation peak at 400 nm (region I) resulted in alkaline-biased fluorescence similar to that described for the wild-type chromophore in vitro, suggesting that wild-type spectral features disrupted in vitro by mutagenesis may be recovered in intact cells. Calibration of intracellular pH (pHi) with in situ fluorescence following excitation in either region revealed a semilogarithmic relationship between fluorescence intensity and pH within the physiologic range. We therefore measured pHi changes attributable to altered Na/HCO3 cotransport (NBC) activity both in GFPmut1-expressing cells and in paired untransfected cells loaded with BCECF. Basal NBC activity was the same in each group, as was the stimulation of activity by 10% CO2, thus validating the utility of GFPmut1 as a fluorescent probe for pHi and establishing a novel, useful, and practical application for GFPmut1 in monitoring pHi in real time.

L12 ANSWER 14 OF 17 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1998284012 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9618493
TITLE: Measurement of cytosolic, mitochondrial, and Golgi pH in single living cells with green fluorescent proteins.
AUTHOR: Llopis J; McCaffery J M; Miyawaki A; Farquhar M G; Tsien R Y
CORPORATE SOURCE: Department of Pharmacology, University of California at San Diego, La Jolla, CA 92093-0647, USA.
CONTRACT NUMBER: CA 58689 (NCI)
NS 27177 (NINDS)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Jun 9) Vol. 95, No. 12, pp. 6803-8.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 16 Jul 1998
Last Updated on STN: 16 Jul 1998
Entered Medline: 9 Jul 1998

AB Many cellular events depend on a tightly compartmentalized distribution of H+ ions across membrane-bound organelles. However, measurements of organelle pH in living cells have been scarce. Several mutants of the Aequorea victoria green fluorescent protein (GFP) displayed a pH-dependent absorbance and fluorescent emission, with apparent pKa values ranging from 6.15 (mutations F64L /S65T/H231L) and 6.4 (K26R/F64L/S65T/Y66W/N146I/M153T/ V163A/N164H/H231L) to a remarkable 7.1 (S65G/S72A/T203Y/H231L). We have targeted these GFPs to the cytosol plus nucleus, the medial/trans-Golgi by fusion with galactosyltransferase, and the mitochondrial matrix by using the targeting signal from subunit IV of cytochrome c oxidase. Cells in culture transfected with these cDNAs displayed the expected subcellular localization by light and electron microscopy and reported local pH that was calibrated in situ with ionophores. We monitored cytosolic and nuclear pH of HeLa cells, and mitochondrial matrix pH in HeLa cells and in rat neonatal cardiomyocytes. The pH of the medial/trans-Golgi was measured at steady-state (calibrated to be 6.58 in HeLa cells) and after various manipulations. These demonstrated that the Golgi membrane in intact cells is relatively permeable to H+, and that Cl- serves as a counter-ion for H+ transport and likely helps to maintain electroneutrality. The amenability to engineer GFPs to specific subcellular locations or tissue targets using gene fusion and transfer techniques should allow us to examine pH at sites previously inaccessible.

L12 ANSWER 15 OF 17 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 6
ACCESSION NUMBER: 1997-05862 BIOTECHDS
TITLE: New fluorescent proteins with increased intensity; jellyfish or Renilla reniformis green fluorescent protein mutagenesis and expression in e.g. Escherichia coli for enhanced fluorescence
AUTHOR: Thastrup O; Tullin S; Poulsen L K; Bjorn S P
PATENT ASSIGNEE: Novo-Nordisk
LOCATION: Bagsvaerd, Denmark.
PATENT INFO: WO 9711094 27 Mar 1997
APPLICATION INFO: WO 1996-DK51 31 Jan 1996
PRIORITY INFO: DK 1995-1065 22 Sep 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1997-202813 [18]
AB A fluorescent protein (I) derived from jellyfish (Aequorea victoria) or Renilla reniformis green fluorescent protein (GFP) or any functional analog is new in which the amino acid in position-1 preceding the chromophore is mutated to increase fluorescence intensity. Also claimed are: a fusion compound containing a GFP linked to a protein; a DNA sequence encoding (I) or a fusion compound; a DNA construct containing a suitable control region or regions and a DNA sequence under the control of the control region; and a host cell (e.g. Escherichia coli) transformed with the DNA construct. (I) can be used as a reporter for organelles or cell processes in living cells. It can be used to measure metabolic, protein kinase or dephosphorylation activity. (I) may also be used as a selectable marker and for bacterial detection. In an example, total RNA isolated from a jellyfish was used to prepare DNA which was subjected to polymerase chain reaction to obtain GFP DNA. The DNA was mutagenized to obtain DNA encoding mutants Y66H-GFP, F64L-GFP, F64L-S65T-GFP and F64L-Y66H-GFP. The F64L mutation enhanced fluorescence. (45pp)

L12 ANSWER 16 OF 17 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP.. on STN
DUPLICATE 7
ACCESSION NUMBER: 1997-09747 BIOTECHDS
TITLE: An improved GFP cloning cassette designed for prokaryotic transcriptional fusions; green fluorescent protein mutant reporter gene expression in Escherichia coli or Pseudomonas spp., using a plasmid pGreenTIR vector with a new DNA cassette
AUTHOR: Miller W G; *Lindow S E
CORPORATE SOURCE: Univ.California
LOCATION: Department of Plant and Microbial Biology, University of California, 111 Koshland Hall, Berkeley, CA 94720, USA. Email: icelab@violet.berkeley.edu
SOURCE: Gene; (1997) 191, 2, 149-53
CODEN: GENED6
ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A new Aequorea victoria green fluorescent protein (GFP) cloning DNA cassette (in a plasmid pGreenTIR vector) was designed for prokaryote transcriptional fusions, e.g. in Escherichia coli. The cassette consisted of the GFP gene (with a S65T red-shift mutation (moving the excitation maximum from 395 to 490 nm) and F64L protein solubility mutations) flanked by 8 restriction sites, a translational enhancer and a consensus ribosome binding site, with an optimized spacer region. GFP fusion strains with this cassette showed 40- to 80-fold greater fluorescence intensity than wild-type GFP fusion strains. The cassette conferred sufficient fluorescence to

recipients to be used in low plasmid copy number vectors, with promoters conferring low transcription levels, and in bacterial taxa other than Escherichia spp., e.g. Pseudomonas spp. (15 ref)

L12 ANSWER 17 OF 17 LIFESCI COPYRIGHT 2007 CSA on STN
ACCESSION NUMBER: 1999:44781 LIFESCI
TITLE: Modified green fluorescent proteins
AUTHOR: Tsien, R.Y.; Heim, R.
CORPORATE SOURCE: The Regents of the University of California
SOURCE: (19970429) . US Patent 5625048; US Class: 536/23.4; 435/6; 536/23.5..
DOCUMENT TYPE: Patent
FILE SEGMENT: W3
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Modifications in the sequence of Aequorea wild-type GFP provide products having markedly different excitation and emission spectra from corresponding products from wild-type GFP. In one class of modifications, the product derived from the modified GFP exhibits an alteration in the ratio of two main excitation peaks observed with the product derived from wild-type GFP. In another class, the product derived from the modified GFP fluoresces at a shorter wavelength than the corresponding product from wild-type GFP. In yet another class of modifications, the product derived from the modified GFP exhibits only a single excitation peak and enhanced emission relative to the product derived from wild-type GFP. Disclosed are synthetic and "humanized" versions of green fluorescent protein (GFP) genes adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such humanized genes are also disclosed. In addition, various methods for using the efficient expression of humanized GFP in mammalian cells and in animals are described. Three classes of GFP mutants having single excitation maxima around 488 nm are brighter than lid-type GFP following 488 nm excitation. GFPmut1 has a double substitution: F64L, S65T; GFPmut2 has a triple substitution: S65A, V68L, S72A; and GFPmut3 is characterized by the double substitution S65G, S72A. The excitation maxima of the three mutants are at 488 nm, 481 nm and 501 nm respectively. The fluorescence intensities following excitation at 488 nm are an order of magnitude higher than that of wild-type GFP excited at 488 nm in E. coli. The expression of GFP is observable minutes after induction.

=> d his

(FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007
L1 115659 S GREEN (W)FLUORESCENT
L2 22 S AEQUOREA (W) COERULESCENS
L3 13 S L1 AND L2
L4 7 DUP REM L3 (6 DUPLICATES REMOVED)
L5 5 S "ACGFPL"
L6 20 S "E222G" AND L1
L7 12 DUP REM L6 (8 DUPLICATES REMOVED)
L8 4 S L2 AND L6
L9 302 S V11I OR K101E OR 1206A OR F64L
L10 3003 S AEQUOREA AND L1
L11 35 S L9 AND L10
L12 17 DUP REM L11 (18 DUPLICATES REMOVED)

=> dup rem l2
PROCESSING COMPLETED FOR L2

L13 13 DUP REM L2 (9 DUPLICATES REMOVED)

=> d 1-13 ibib ab

L13 ANSWER 1 OF 13 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-19750 BIOTECHDS
TITLE: Novel transgenic zebrafish that expresses Tau, amyloid precursor protein or presenilin polypeptide or their fusion polypeptides, or amyloid beta polypeptide, useful for identifying modulator of Alzheimer's disease;
tau protein, amyloid precursor protein, amyloid-beta or presenilin gene transfer and expresion in zebrafish neuron for zebrafish transgenic fish and drug screening
AUTHOR: RUBINSTEIN A L
PATENT ASSIGNEE: ZYGOGEN LLC
PATENT INFO: WO 2006081539 3 Aug 2006
APPLICATION INFO: WO 2006-US3165 27 Jan 2006
PRIORITY INFO: US 2005-647493 27 Jan 2005; US 2005-647493 27 Jan 2005
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2006-539425 [55]
AB DERWENT ABSTRACT:
NOVELTY - A transgenic zebrafish that expresses (a) a tau polypeptide, amyloid precursor protein (APP), amyloid beta or presenilin polypeptide, comprising a zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a tau, APP, amyloid beta or presenilin polypeptide, which is expressed in the neurons of the transgenic zebrafish, where the transgenic zebrafish exhibits a pathology associated with Alzheimer's Disease, or (b) a tau, APP or presenilin fusion polypeptide, comprising a zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a fusion polypeptide comprising a tau, APP or presenilin polypeptide and a fluorescent reporter polypeptide, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for: 1) a transgenic zebrafish that expresses a Tau (fusion) polypeptide; 2) a transgenic zebrafish that expresses an APP (fusion) polypeptide;3) a transgenic zebrafish that expresses an amyloid beta polypeptide;4) a transgenic zebrafish that expresses a presenilin (fusion) polypeptide;

BIOTECHNOLOGY - Preferred Zebrafish: The zebrafish further comprises zebrafish neuron specific expression sequence operably linked to a nucleic acid encoding a fluorescent reporter polypeptide e.g. green fluorescent protein (GFP), Aequorea coerulescens green fluorescent protein (AcGFP) and DsRedExpress (DsRed protein). The neuron specific expression sequence is a neuron-specific promoter chosen from an elav promoter and a GATA-2 promoter. The zebrafish neuron specific expression sequence and the sequence encoding the tau, APP, amyloid beta polypeptide are contained in an exogenous construct. The zebrafish develops neurofibrillary tangles, or exhibits neuronal cell damage. The tau, APP polypeptide, amyloid beta or presenilin is a mutant tau, APP, amyloid beta polypeptide or presenilin. The expression sequence comprises an inducible promoter, being an inducible UAS promoter activated by GAL4/VP16. The zebrafish further comprises a nucleic acid encoding a zinc transporter. Preferred Method: Identifying an agent that modulates a pathology associated with disease comprises: a) contacting the zebrafish with a test agent; b) comparing the neuronal pathology of the zebrafish contacted with the test agent to the neuronal pathology of a zebrafish not contacted with the test agent; c) determining the effect of the test agent on the zebrafish, such that if there is a difference in the neuronal pathology of the zebrafish contacted with the test agent and the zebrafish not contacted with the test agent, the test agent is an agent that modulates a pathology associated with Alzheimer's disease. The difference in neuronal pathology is a decrease in neuronal cell death in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent or a decrease in neurofibrillary

tangles in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent. The difference is neuronal pathology is a decrease in neuronal fluorescence. The difference in neuronal pathology is a decrease in protein expression in the zebrafish contacted with the test agent as compared to the zebrafish not contacted with the test agent. Identifying an agent that modulates neuronal pathology comprises: a) administering a test agent to a transgenic zebrafish expressing a reporter protein in neurons; b) comparing the expression of the reporter protein in the neurons of the zebrafish contacted with the test agent with the expression of the reporter protein in the neurons of a transgenic zebrafish that was not contacted with the test agent; and c) determining the effect of the test compound on the expression of the reporter protein in the neurons, such that if the number of neurons in the zebrafish contacted with the test agent is greater than the number of neurons in the zebrafish that was not contacted with the test agent, the test agent is an agent that modulates neuronal pathology and is a neuroproliferative agent. The reporter protein is a fluorescent reporter polypeptide.

ACTIVITY - Nootropic; Neuroprotective. No biological data given.

MECHANISM OF ACTION - None given.

USE - For identifying an agent that modulates a pathology associated with Alzheimer's disease (claimed).

ADVANTAGE - The transgenic zebrafish enables identification of an agent that modulates a pathology associated with Alzheimer's disease.(75 pages)

L13 ANSWER 2 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 2007:30318 BIOSIS
DOCUMENT NUMBER: PREV200700030032
TITLE: The 1.7 angstrom crystal structure of Dronpa: A photoswitchable green fluorescent protein.
AUTHOR(S): Wilmann, Pascal G.; Turcic, Kristina; Battad, Jion M.; Wilce, Matthew C. J.; Devenish, Rodney J.; Prescott, Mark [Reprint Author]; Rossjohn, Jamie
CORPORATE SOURCE: Monash Univ, Prot Crystallog Unit, Clayton, Vic 3800, Australia
Mark.Prescott@med.monash.edu.au; Jamie.Rossjohn@med.monash.edu.au
SOURCE: Journal of Molecular Biology, (NOV 24 2006) Vol. 364, No. 2, pp. 213-224.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Dec 2006
Last Updated on STN: 27 Dec 2006

AB The green fluorescent protein (GFP), its variants, and the closely related GFP-like proteins possess a wide variety of spectral properties that are of widespread interest as biological tools. One desirable spectral property termed photoswitching, involves the light-induced alteration of the optical properties of certain GFP members. Although the structural basis of both reversible and irreversible photoswitching events have begun to be unraveled, the mechanisms resulting in reversible photoswitching are less clear. A novel GFP-like protein, Dronpa, was identified to have remarkable light-induced photoswitching properties, maintaining an almost perfect reversible photochromic behavior with a high fluorescence to dark state ratio. We have crystallized and subsequently determined to 1.7 angstrom resolution the crystal structure of the fluorescent state of Dronpa. The chromophore was observed to be in its anionic form, adopting a cis co-planar conformation. Comparative structural analysis of non-photoactivatable and photoactivatable GFPs, together with site-directed mutagenesis of a position (Cys62) within the Dronpa chromophore, has provided a basis for understanding Dronpa photoactivation. Specifically, we propose a model of reversible photoactivation whereby irradiation with light leads to subtle

conformational changes within and around the environment of the chromophore that promotes proton transfer along an intricate polar network. (c) 2006 Elsevier Ltd. All rights reserved.

L13 ANSWER 3 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:456583 BIOSIS
DOCUMENT NUMBER: PREV200600457279
TITLE: The kindling fluorescent protein: A transient photoswitchable marker.
AUTHOR(S): Henderson, J. Nathan [Reprint Author]; Remington, S. James
CORPORATE SOURCE: Univ Oregon, Dept Chem, Eugene, OR 97403 USA jremington@uoxray.uoregon.edu
SOURCE: Physiology (Bethesda), (JUN 2006) Vol. 21, pp. 162-170. ISSN: 1548-9213.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Sep 2006
Last Updated on STN: 13 Sep 2006

AB Passive fluorescent protein markers are indispensable for dynamic cellular imaging; however, they are unselective, introduce constant background flourescence, and require continuous observation. Photoactivatable flourescent proteins have now been developed whose fluorescence can be switched on and off by illumination, allowing selective and direct tracking of tagged objects without the need for continuous imaging. The "kindling fluorescent protein" is a photoactivatable marker with a novel twist: it turns itself off after a selectable period.

L13 ANSWER 4 OF 13 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2006483272 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 16909160
TITLE: Practical three color live cell imaging by widefield microscopy.
AUTHOR: Xia Jianrun; Kim Song Hon H; Macmillan Susan; Truant Ray
CORPORATE SOURCE: Department of Biochemistry and Biomedical Sciences, McMaster University, HSC 4H24A 1200 Main Street West, Hamilton, Ontario, L8N 3Z5, Canada.
SOURCE: Biological procedures online, (2006) Vol. 8, pp. 63-8. Electronic Publication: 2006-07-21.
Journal code: 100963717. E-ISSN: 1480-9222.
PUB. COUNTRY: Canada
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-DATA-REVIEW; IN-PROCESS; NONINDEXED
ENTRY DATE: Entered STN: 17 Aug 2006
Last Updated on STN: 12 Dec 2006

AB Live cell fluorescence microscopy using fluorescent protein tags derived from jellyfish and coral species has been a successful tool to image proteins and dynamics in many species. Multi-colored aequorea fluorescent protein (AFP) derivatives allow investigators to observe multiple proteins simultaneously, but overlapping spectral properties sometimes require the use of sophisticated and expensive microscopes. Here, we show that the aequorea coerulescens fluorescent protein derivative, PS-CFP2 has excellent practical properties as a blue fluorophore that are distinct from green or red fluorescent proteins and can be imaged with standard filter sets on a widefield microscope. We also find that by widefield illumination in live cells, that PS-CFP2 is very photostable. When fused to proteins that form concentrated puncta in either the cytoplasm or nucleus, PSCFP2 fusions do not artifactually interact with other AFP fusion proteins, even at very high levels of over-expression. PSCFP2 is therefore a good blue fluorophore for distinct three color imaging along with eGFP and mRFP using a relatively simple and inexpensive microscope.

L13 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:660610 HCAPLUS
DOCUMENT NUMBER: 143:130107
TITLE: Preparation of transgenic theaceous plants
INVENTOR(S): Tanba, Yasuo; Kato, Michiyo
PATENT ASSIGNEE(S): Hamamatsu Foundation for Science and Technology Promotion, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 16 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2005198600	A	20050728	JP 2004-9649	20040116
PRIORITY APPLN. INFO.:			JP 2004-9649	20040116

AB The fluorescent heterologous gene(s) is introduced specifically into organelles of the embryonic callus for easy and non-invasive selection of transgenic theaceous plants. The organelles are selected from mitochondria and plastid. The fluorescence is obtained from the (mutated) green fluorescence protein, especially that of Aequorea coerulescens. Transformation of tea tree using mitochondria- and plastid-specific binary vectors was shown. Also given was the construction of mitochondria- and plastid-specific binary vectors using 35Ω-sGFP plasmid containing CMV 35S promoter, sGFP (S65T) gene, nopaline synthase polyadenylation signal, etc.

L13 ANSWER 6 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:490521 BIOSIS
DOCUMENT NUMBER: PREV200510282648
TITLE: Immunochemical recognition of gelatinous zooplankton: an application to identify the origin of the 'barrel' made by the pelagic amphipod, Phronima sedentaria.
AUTHOR(S): Nishikawa, Jun [Reprint Author]; Suzuki, Yuzuru; Nishida, Shuhei
CORPORATE SOURCE: Univ Tokyo, Ocean Res Inst, Nakano Ku, Tokyo 1648639, Japan
jn@ori.u-tokyo.ac.jp
SOURCE: Journal of the Marine Biological Association of the United Kingdom, (JUN 2005) Vol. 85, No. 3, pp. 635-639.
CODEN: JMBAAK. ISSN: 0025-3154.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Nov 2005
Last Updated on STN: 16 Nov 2005

AB A first trial to recognize gelatinous zooplankton using an immunochemical method was carried out. Polyclonal antibodies (rabbit IgG) raised against three cnidarians and four pelagic tunicates were purified and tested for their specificity against various sympatric zooplankton and micronekton. While antibodies to cnidarians were species-specific or at least distinguishable by means of the patterns of the bands appearing, antibodies to the pelagic tunicates cross-reacted with the antigen from the other tunicate species although these did not cross-react with other animal taxa. These results suggest that the present cnidarian antibodies can be applied to the predator identification at the species level. Antibodies to pelagic tunicates would also be effective tools for basic scanning at higher taxonomic levels, i.e. pelagic tunicates or other animals. The antibodies were applied to the identification of the 'barrel' of Phronima, indicating that the 'barrels' originated from tunicates but not From cnidarians. The present study indicates that the immunochemical method using polyclonal antibodies is a powerful tool for the detection of gelatinous zooplankton.

L13 ANSWER 7 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:417169 BIOSIS
DOCUMENT NUMBER: PREV200400418817
TITLE: Two new records of Hydromedusae (Cnidaria: Hydrozoa) in Korea.
AUTHOR(S): Park, Jung Hee [Reprint Author]; Song, Jun-Im
CORPORATE SOURCE: Coll Nat SciDept Life Sci, Univ Suwon, Kyonggi Do, 45743, South Korea
jhpark5@suwon.ac.kr
SOURCE: Korean Journal of Systematic Zoology, (April 2004) Vol. 20, No. 1, pp. 31-37. print.
ISSN: 1018-192X (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Oct 2004
Last Updated on STN: 27 Oct 2004

AB Some hydromedusae were collected from the coasts of Seogwipo (Jejudo Island), Hoenggando Island and Ilsanhaesuyokjang (Ulsan), Korea on July 11, 1985; July 23, 1990 and July 16, 1994. They were identified into Aequorea coerulescens (Brandt, 1838) of the order Leptomedusae, and Physalia physalis utriculus La Martiniere, 1829 of the Siphonophora, respectively. The unique morphological characteristics of A. coerulescens are smooth even surface of exumbrella, large mouth with 60 highly fringed oral lobes, shallow stomach, 120 simple radial canals and flat beret-shaped bell. In P. physalis utriculus its morphological characteristics are a triangular large pneumatophore with very low or rudimentary crest, a ribbon like long slender main tentacle, siphon-shaped gastrozooid with mouth, finger-shaped dactylozooid and branched gonozooid with gonophores. P. physalia. utriculus is the Pacific form and distinguished from the Atlantic form, P. p. physalis which has a much larger pneumatophore with high crest, numerous large main tentacles, and compact arrangement of basal and ventral cormidia. As a result of this work the Korean hydromedusan fauna consists of 15 species of five orders.

L13 ANSWER 8 OF 13 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2003-22532 BIOTECHDS
TITLE: New nucleic acid molecule present in other than its natural environment and that encodes a fluorescent protein from Aequorea coerulescens, useful for various labeling applications;
involving vector-mediated gene transfer and expression in host cell for use in labeling and biosensor
AUTHOR: GURSKAYA N; FRADLOV A; LUKYANOV S; PUNKOVA N
PATENT ASSIGNEE: EVROGEN JSC
PATENT INFO: WO 2003062270 31 Jul 2003
APPLICATION INFO: WO 2003-IB907 17 Jan 2003
PRIORITY INFO: US 2002-351518 22 Jan 2002; US 2002-351518 22 Jan 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-608187 [57]

AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid molecule present in other than its natural environment and that encodes a fluorescent protein from Aequorea coerulescens, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a construct comprising a vector and the above nucleic acid molecule; (2) an expression cassette comprising a transcriptional initiation region functional in an expression host, the above nucleic acid molecule, and a transcriptional termination region functional in the expression host; (3) a cell, or its progeny, comprising the expression cassette; (4) a method of producing a chromo- or fluorescent protein, comprising growing the cell cited above under conditions where the chromo- or fluorescent protein is expressed; (5) a protein or its

fragment encoded by the above nucleic acid, or a protein or its fragment having a sequence similarity of at least about 95% to the above-mentioned protein or fragment; (6) a fusion protein incorporating the protein or fragment cited above; (7) an antibody binding specifically to the above protein; (8) a transgenic organism comprising the above nucleic acid; and (9) a kit comprising the above nucleic acid and instructions for using the nucleic acid.

BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid is isolated. It encodes a fluorescent protein comprising any of the 12 amino acid sequences not clearly defined in the specification. The nucleic acid comprises a sequence that is substantially similar to or identical to a nucleotide sequence of at least 10 residues in length taken from any of the 12 nucleotide sequences not clearly defined in the specification. Alternatively, the nucleic acid has a sequence similarity of at least about 70% with any of the above-mentioned nucleotide sequences. Additionally, the nucleic acid encodes a mutant fluorescent protein comprising at least one point mutation or at least one deletion mutation as compared to a wild-type protein. The nucleic acid or its mimetic may hybridize under stringent conditions to a similar nucleic acid or its complements or fragments. Preferred Method: Producing a chromo- or fluorescent protein further comprises isolating the chromo- or fluorescent protein substantially free of other proteins. Preparation: The nucleic acid molecule was prepared using standard isolation techniques.

USE - The nucleic acid molecule and protein are useful in labeling applications, in fluorescence resonance energy transfer methods, or as biosensors in prokaryotic and eukaryotic cells. (76 pages)

L13 ANSWER 9 OF 13 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2003313870 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12693991
TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.
AUTHOR: Gurskaya Nadya G; Fradkov Arkady F; Pounkova Natalia I; Staroverov Dmitry B; Bulina Maria E; Yanushevich Yurii G; Labas Yulii A; Lukyanov Sergey; Lukyanov Konstantin A
CORPORATE SOURCE: Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16/10, Moscow 117997, Russia.
SOURCE: The Biochemical journal, (2003 Jul 15) Vol. 373, No. Pt 2, pp. 403-8.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY151052; GENBANK-AY233272
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 8 Jul 2003
Last Updated on STN: 16 Aug 2003
Entered Medline: 15 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222)-->Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Re-introduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced

photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines. Furthermore, expression of a fusion protein of 'humanized' aceGFP and beta-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

L13 ANSWER 10 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 2001:406047 BIOSIS
DOCUMENT NUMBER: PREV200100406047
TITLE: Occurrence of organo-arsenicals in jellyfishes and their mucus.
AUTHOR(S): Hanaoka, Ken'ichi [Reprint author]; Ohno, Hirokazu; Wada, Namiko; Ueno, Shunshiro; Goessler, Walter; Kuehnelt, Doris; Schlagenhaufen, Claudia; Kaise, Toshikazu; Irgolic, K. J.
CORPORATE SOURCE: Department of Food Science and Technology, National Fisheries University, Nagata honmachi 2-7-1, Shimonoseki, 759-65, Japan
SOURCE: Chemosphere, (August, 2001) Vol. 44, No. 4, pp. 743-749. print.
CODEN: CMSHAF. ISSN: 0045-6535.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Aug 2001
Last Updated on STN: 22 Feb 2002

AB Water-soluble arsenic compound fractions were extracted from seven species of jellyfishes and subjected to analysis by high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) for arsenicals. A low content of arsenic was found to be the characteristic of jellyfish. Arsenobetaine (AB) was the major arsenic compound without exception in the tissues of the jellyfish species and mucus-blobs collected from some of them. Although the arsenic content in Beroe cucumis, which preys on Bolinopsis mikado, was more than 13 times that in B. mikado, the chromatograms of these two species were similar in the distribution pattern of arsenicals. The nine species of jellyfishes including two species treated in the previous paper can be classified into arsenocholine (AC)-rich and AC-poor species. Jellyfishes belonging to Semaostamae were classified as AC-rich species.

L13 ANSWER 11 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:22775 BIOSIS
DOCUMENT NUMBER: PREV200000022775
TITLE: The first finding of hydroid medusae of the genus Aequorea in Russian waters of the Sea of Japan.
AUTHOR(S): Pogodin, A. G. [Reprint author]; Yakovlev, Yu. M. [Reprint author]
CORPORATE SOURCE: Far East Branch, Institute of Marine Biology, Russian Academy of Sciences, Vladivostok, 690041, Russia
SOURCE: Biologiya Morya (Vladivostok), (Sept.-Oct., 1999) Vol. 25, No. 5, pp. 389-392. print.
CODEN: BIMOD4. ISSN: 0134-3475.
DOCUMENT TYPE: Article
LANGUAGE: Russian
ENTRY DATE: Entered STN: 29 Dec 1999
Last Updated on STN: 31 Dec 2001

AB A large-sized hydrozoan jellyfish Aequorea coerulescens (Brandt, 1838) (Aequoreidae, Thecaphora, Hydrozoa) is recorded in Russian waters of the northwestern Sea of Japan for the first time. Medusae were captured on 22-30 September 1995 in the neritic zone of northern Primorye between 43 and 45degree N using a large conical net and scuba diving. A description of details of the body and measurements are given. The umbrella is 16 to 140 mm in diameter.

L13 ANSWER 12 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1967:51904 BIOSIS
DOCUMENT NUMBER: PREV19674800051904; BA48:51904
TITLE: Life cycle of a hydrozoan, Campanulina type or Aequorea coerulescens Brandt.
AUTHOR(S): KAKINUMA, YOSHIKO
CORPORATE SOURCE: Mar. Biol. Sta. Asamushi, Tohoku Univ., Aomori City, Jap.
SOURCE: BULL MAR BIOL STA ASAMUSHI TOHOKU UNIV, (1966) Vol. 12, No. 4, pp. 211-218.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: Unavailable
ENTRY DATE: Entered STN: May 2007
Last Updated on STN: May 2007
AB A hydrozoan colony of Campanulina type was cultivated in the laboratory and the development of the medusa for two and a half months was investigated. The present species is identified as Aquorea coerulescens Brandt, and the life cycle is described for the first time in this paper. ABSTRACT AUTHORS: Author

L13 ANSWER 13 OF 13 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1928:14107 BIOSIS
DOCUMENT NUMBER: PREV19280200014215; BA02:14215
TITLE: Report of the biological survey of Mutsu Bay. Medusae of Mutsu Bay.
AUTHOR(S): UCHIDA, TOHRU
SOURCE: SCI REPT TOHOKU IMP UNIV 4TH SER BIOL, (1927) Vol. 2, No. 3, pp. 215-238.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: Unavailable
ENTRY DATE: Entered STN: May 2007
Last Updated on STN: May 2007
AB Lists 14 species of Hydromedusae, 6 Scyphomedusae, and 2 Ctenophorae. Melicertum campanula and Aequorea coerulescens newly recorded in Japan; Rhopilema esculenta v. asamushi. ABSTRACT AUTHORS: T. Uchida

=> d his

(FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007
L1 115659 S GREEN (W)FLUORESCENT
L2 22 S AEQUOREA (W) COERULESCENS
L3 13 S L1 AND L2
L4 7 DUP REM L3 (6 DUPLICATES REMOVED)
L5 5 S "ACGFPL"
L6 20 S "E222G" AND L1
L7 12 DUP REM L6 (8 DUPLICATES REMOVED)
L8 4 S L2 AND L6
L9 302 S V11I OR K101E OR 1206A OR F64L
L10 3003 S AEQUOREA AND L1
L11 35 S L9 AND L10
L12 17 DUP REM L11 (18 DUPLICATES REMOVED)
L13 13 DUP REM L2 (9 DUPLICATES REMOVED)

=> e gurskaya n g/au
E1 1 GURSKAYA M P/AU

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E2          12      GURSKAYA N/AU
E3          79 --> GURSKAYA N G/AU
E4          10      GURSKAYA N I/AU
E5          10      GURSKAYA N V/AU
E6          41      GURSKAYA N Z/AU
E7           2      GURSKAYA NADEJDA/AU
E8           3      GURSKAYA NADEZDA GEORGIEVNA/AU
E9           3      GURSKAYA NADIA/AU
E10          2      GURSKAYA NADIA G/AU
E11          3      GURSKAYA NADYA/AU
E12         37      GURSKAYA NADYA G/AU

=> s e3-e7
L14        142 ("GURSKAYA N G"/AU OR "GURSKAYA N I"/AU OR "GURSKAYA N V"/AU OR
               "GURSKAYA N Z"/AU OR "GURSKAYA NADEJDA"/AU)

=> e fradkov a f/au
E1           2      FRADKOV A A/AU
E2          39      FRADKOV A B/AU
E3         103 --> FRADKOV A F/AU
E4           5      FRADKOV A I/AU
E5         112      FRADKOV A L/AU
E6           9      FRADKOV A S/AU
E7           2      FRADKOV ALEXANDER/AU
E8           3      FRADKOV ALEXANDER L/AU
E9           6      FRADKOV ARCADY/AU
E10          2      FRADKOV ARCADY F/AU
E11         15      FRADKOV ARCADY FEDOROVICH/AU
E12          9      FRADKOV ARKADY/AU

=> s e3 or e12
L15        112 "FRADKOV A F"/AU OR "FRADKOV ARKADY"/AU

=> e lukyanov s a/au
E1           1      LUKYANOV RS/AU
E2         236      LUKYANOV S/AU
E3         201 --> LUKYANOV S A/AU
E4           2      LUKYANOV S A */AU
E5           1      LUKYANOV S G/AU
E6          18      LUKYANOV S I/AU
E7          21      LUKYANOV S L/AU
E8         265      LUKYANOV S M/AU
E9           3      LUKYANOV S N/AU
E10          4      LUKYANOV S P/AU
E11          1      LUKYANOV S S/AU
E12         24      LUKYANOV S V/AU

=> s e3
L16        201 "LUKYANOV S A"/AU

=> e punkova n i/au
E1           1      PUNKOVA N/AU
E2           1      PUNKOVA N G/AU
E3           1 --> PUNKOVA N I/AU
E4           1      PUNKOVA N L/AU
E5           1      PUNKOVA NATALIA/AU
E6           3      PUNKOVA NATALIA I/AU
E7           1      PUNKOVIC N/AU
E8           1      PUNKOVSKII A N/AU
E9           8      PUNKRUT W/AU
E10         43      PUNKT J/AU
E11          7      PUNKT JUERGEN/AU
E12         97      PUNKT K/AU
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L17           3 "PUNKOVA NATALIA I"/AU

=> d his

     (FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007
L1         115659 S GREEN (W)FLUORESCENT
L2             22 S AEQUOREA (W) COERULESCENS
L3             13 S L1 AND L2
L4              7 DUP REM L3 (6 DUPLICATES REMOVED)
L5              5 S "ACGFPL"
L6             20 S "E222G" AND L1
L7             12 DUP REM L6 (8 DUPLICATES REMOVED)
L8              4 S L2 AND L6
L9            302 S V11I OR K101E OR 1206A OR F64L
L10          3003 S AEQUOREA AND L1
L11            35 S L9 AND L10
L12            17 DUP REM L11 (18 DUPLICATES REMOVED)
L13            13 DUP REM L2 (9 DUPLICATES REMOVED)
                  E GURSKAYA N G/AU
L14           142 S E3-E7
                  E FRADKOV A F/AU
L15           112 S E3 OR E12
                  E LUKYANOV S A/AU
L16           201 S E3
                  E PUNKOVA N I/AU
L17             3 S E6

=> s l14 or l15 or 116 or 117
L18      269981 L14 OR L15 OR 116 OR 117

=> s l2 and l18
L19             3 L2 AND L18

=> dup rem l19
PROCESSING COMPLETED FOR L19
L20             2 DUP REM L19 (1 DUPLICATE REMOVED)

=> d 1-2 ibib ab

L20  ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         2003:591209  HCAPLUS
DOCUMENT NUMBER:          139:129175
TITLE:                    Sequences of novel fluorescent proteins from Aequorea
                          coerulscens and use
INVENTOR(S):              Gurskaya, Nadejda; Fradlov, Arkadiy;
                          Lukyanov, Sergey; Punkova, Natalia
PATENT ASSIGNEE(S):       Evrogen, Jsc, USA
SOURCE:                   PCT Int. Appl., 76 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
     WO 2003062270         A2    20030731    WO 2003-IB907           20030117
     WO 2003062270         A3    20031127
     WO 2003062270         B1    20040401
     WO 2003062270         A8    20041104
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W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

CA 2474108	A1	20030731	CA 2003-2474108	20030117
EP 1485481	A2	20041215	EP 2003-706812	20030117
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2005526495	T	20050908	JP 2003-562147	20030117
US 2006167225	A1	20060727	US 2004-501629	20040715
PRIORITY APPLN. INFO.:			US 2002-351518P P	20020122
			WO 2003-IB907 W	20030117

AB The present invention provides protein and cDNA sequences of a novel colorless GFP-like protein, acGFP, from Aequorea coerulscens and fluorescent and non-fluorescent mutants and derivs. thereof, as well as peptides and proteins encoded by these nucleic acid compns. The subject protein and nucleic acid compns. of the present invention are colored and/or fluorescent and/or can be photoactivated, and can be used in a variety of different biol. applications, particularly for labeling. Finally, kits for use in such biol. applications are provided.

L20 ANSWER 2 OF 2 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 1

ACCESSION NUMBER: 2003300328 EMBASE

TITLE: A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants.

AUTHOR: Gurskaya N.G.; Fradkov A.F.; Pounkova N.I.; Staroverov D.B.; Bulina M.E.; Yanushevich Y.G.; Labas Y.A.; Lukyanov S.; Lukyanov K.A.

CORPORATE SOURCE: K.A. Lukyanov, Shemyakin/Ovchinnikov Inst. B., Miklukho-Maklaya 16/10, Moscow 117997, Russian Federation. kluk@ibch.ru

SOURCE: Biochemical Journal, (15 Jul 2003) Vol. 373, No. 2, pp. 403-408. .
Refs: 25
ISSN: 0264-6021 CODEN: BIJOAK

COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 14 Aug 2003
Last Updated on STN: 14 Aug 2003

AB We have cloned an unusual colourless green fluorescent protein (GFP)-like protein from Aequorea coerulescens (acGFPL). The A. coerulescens specimens displayed blue (not green) luminescence, and no fluorescence was detected in these medusae. Escherichia coli expressing wild-type acGFPL showed neither fluorescence nor visible coloration. Random mutagenesis generated green fluorescent mutants of acGFPL, with the strongest emitters found to contain an Glu(222) → Gly (E222G) substitution, which removed the evolutionarily invariant Glu(222). Reintroduction of Glu(222) into the most fluorescent random mutant, named aceGFP, converted it into a colourless protein. This colourless aceGFP-G222E protein demonstrated a novel type of UV-induced photoconversion, from an immature non-fluorescent form into a green fluorescent form. Fluorescent aceGFP may be a useful biological tool, as it was able to be expressed in a number of mammalian cell lines.

Furthermore, expression of a fusion protein of 'humanized' aceGFP and β-actin produced a fluorescent pattern consistent with actin distribution in mammalian cells.

=> d his

(FILE 'HOME' ENTERED AT 10:16:17 ON 29 JUN 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:20:39 ON 29 JUN 2007

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L1        115659 S GREEN (W)FLUORESCENT
L2            22 S AEQUOREA (W) COERULESCENS
L3            13 S L1 AND L2
L4             7 DUP REM L3 (6 DUPLICATES REMOVED)
L5             5 S "ACGFPL"
L6            20 S "E222G" AND L1
L7            12 DUP REM L6 (8 DUPLICATES REMOVED)
L8             4 S L2 AND L6
L9           302 S V11I OR K101E OR 1206A OR F64L
L10         3003 S AEQUOREA AND L1
L11           35 S L9 AND L10
L12           17 DUP REM L11 (18 DUPLICATES REMOVED)
L13           13 DUP REM L2 (9 DUPLICATES REMOVED)
                 E GURSKAYA N G/AU
L14          142 S E3-E7
                 E FRADKOV A F/AU
L15          112 S E3 OR E12
                 E LUKYANOV S A/AU
L16          201 S E3
                 E PUNKOVA N I/AU
L17            3 S E6
L18       269981 S L14 OR L15 OR 116 OR 117
L19            3 S L2 AND L18
L20            2 DUP REM L19 (1 DUPLICATE REMOVED)
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	Document ID	Kind Codes	Source	Issue Date	Pages	Title
1	US 20070072267 A1		US-PGPUB	20070329	26	Transformant having galactose induction system and use thereof
2	US 20060257886 A1		US-PGPUB	20061116	12	Method for analyzing molecular fluorescence using evanescent illumination
3	US 20040248208 A1		US-PGPUB	20041209	72	Screening method
4	US 20040171067 A1		US-PGPUB	20040902	89	Screening method
5	US 20040043490 A1		US-PGPUB	20040304	17	Cells to be used in producing virus vector, process for producing the same, and process for producing virus vector with the use of the cells

	Document ID	Kind Codes	Source	Issue Date	Pages	Title
1	US 20070072267 A1		US-PGPUB	20070329	26	Transformant having galactose induction system and use thereof
2	US 20070015229 A1		US-PGPUB	20070118	29	Secretory or membrane-binding chimeric protein
3	US 20060257886 A1		US-PGPUB	20061116	12	Method for analyzing molecular fluorescence using evanescent illumination
4	US 20060188890 A1		US-PGPUB	20060824	21	Ubiquitin-based protein interaction assays and related compositions
5	US 20060167225 A1		US-PGPUB	20060727	56	Novel fluorescent protein from aequorea coerulscens and methods for using the same
6	US 20050181453 A1		US-PGPUB	20050818	32	Ubiquitin-based protein interaction assays and related compositions
7	US 20050032132 A1		US-PGPUB	20050210	24	Cancer diagnostics
8	US 20040248208 A1		US-PGPUB	20041209	72	Screening method
9	US 20040171067 A1		US-PGPUB	20040902	89	Screening method
10	US 20040043490 A1		US-PGPUB	20040304	17	Cells to be used in producing virus vector, process for producing the same, and process for producing virus vector with the use of the cells

	Document ID	Kind Codes	Source	Issue Date	Pages	Title
1	US 20060167225 A1		US-PGPUB	20060727	56	Novel fluorescent protein from aequorea coerulscens and methods for using the same

	L #	Hits	Search Text
1	L1	1	"6919186".pn.
2	L2	1	"6096865".pn.
3	L3	19194	green adj fluorescent adj protein$2
4	L5	5	l3 same l4
5	L4	10	aequorea adj coerulescens
6	L6	298	GURSKAYA LUKYANOV FRADKOV
7	L7	1	l4 and l6